COMMUNICATIONS

Functional hyperaemia in soleus muscle of the cat

By T. Forrester and I. J. D. Hamilton, *Institute of Physiology, University of Glasgow, G12 8QQ*

Blood flow through the soleus muscle of the cat has a resting value 3–4 times greater than that through fast muscle, and functional hyperaemia is reported to be reduced (Folkow & Halicka, 1968; Hudlicka, 1968) and sometimes absent (Hilton, Jeffries & Vrbová, 1970). It is generally accepted that alteration in local chemical environment is responsible for functional hyperaemia, therefore it was of interest to examine further certain characteristics of the soleus vascular bed.

In heparinized cats anaesthetized with intra-peritoneal sodium pentobarbitone, the soleus blood supply was isolated. The femoral vein was cannulated and the venous outflow from soleus was passed through an integrating drop counter and then recycled proximally into the femoral vein. Blood pressure was monitored via a cannula in the common carotid artery. The muscle was held isometrically. Supramaximal pulses of 1 msec duration were applied to the soleus nerve at frequencies of 1–20 Hz for periods of 1 min. After each experiment Naphthol green dye was injected to confirm the selective perfusion of soleus.

The resting flow ($n = 6$) was 34 ml./100 g.min ($\pm$ S.E. 3·8) and during stimulation at 1, 5, 10 and 20 Hz was found to be 38 ($\pm$ 3·7), 39 ($\pm$ 2·0), 45 ($\pm$ 3·7) and 57 ($\pm$ 6·6) respectively. It is concluded that the soleus muscle has a high resting blood flow and a reduced functional hyperaemia in agreement with previous workers. Close arterial infusion at 1 ml./min of 10 μM-ATP, adenosine and NaH_2PO_4 into soleus muscles ($n = 6$) produced a 2·1 ($\pm$ S.E. 0·34), 1·6 ($\pm$ 0·17) and 1·4 ($\pm$ 0·26) increase in flow ($\times$ resting flow) respectively. Finally, samples were collected from the soleus muscle perfused with blood or an oxygenated Krebs solution. All blood samples were collected into melting ice and centrifuged at 25000 *g* for 1 min. The cell-free plasma was assayed for ATP using a modification of the firefly technique (Strehler & McElroy, 1957). ATP levels in plasma ($n = 8$) were: resting, 0·07 μM ($\pm$ S.E. 0·04) and during stimulation at 10 Hz, 0·3 μM ($\pm$ 0·05); this difference is probably significant ($0{\cdot}05 > P > 0{\cdot}01$). Corresponding values for muscles perfused with Krebs solution ($n = 12$) were 0·9 μM ($\pm$ 0·26) and 1·8 μM ($\pm$ 0·48); this difference is not significant.

The lower levels of ATP detected in plasma could be due to surface ATP-ase activity of R.B.C.S (Parker, 1970) and the higher levels in the Krebs perfusate might result from the release of ATP by hypoxic muscle.

In conclusion, as the soleus blood vessels are sensitive to ATP, could a continuous release of ATP by soleus account for its high resting blood flow and the reduced functional hyperaemia result from only a modest increase in the release of ATP?

REFERENCES

FOLKOW, B. & HALICKA, H. D. (1968). *Microvasc. Res.* **1**, 1–14.
HILTON, S. M., JEFFRIES, M. G. & VRBOVÁ, G. (1970). *J. Physiol.* **206**, 543–562.
HUDLICKA, O. (1969). *Microvasc. Res.* **1**, 390–402.
STREHLER, B. L. & MCELROY, W. D. (1957). In *Methods in Enzymology*, ch. 3, ed. COLOWICK, S. P. & KAPLAN, N. O., pp. 871–873. New York: Academic Press.
PARKER, J. C. (1970). *Am. J. Physiol.* **218**, 1568–1574.

Non-myelinated nerve X-ray scattering patterns

BY G. R. CLARKE and E. G. STEWARD. *Physics Department, The City University, St John Street, London EC1V 4PB*

X-ray scattering patterns from non-myelinated crab peripheral nerve bundles have been obtained using an Elliott GX6 rotating-anode generator and a Searle small-angle scattering camera with toroid optics. Most X-ray studies on nerve have involved myelinated specimens (Worthington, 1971).

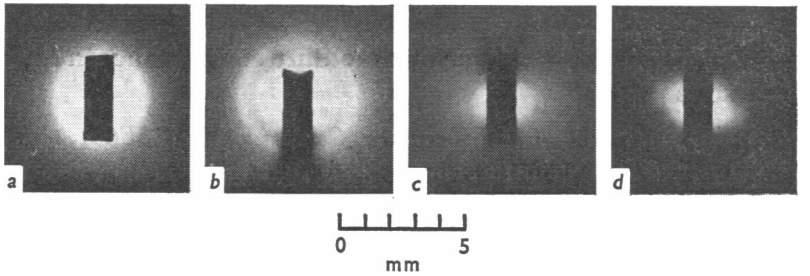

Fig. 1. X-ray scattering patterns from non-myelinated nerve bundles at the following humidities: (*a*) 84%, (*b*) 65%, (*c*) at 55%, and (*d*) at 33%. Radiation CuKα, λ = 1·54 Å; specimen to film distance = 7·0 cm.

Studies on non-myelinated nerve (Schmitt, Bear & Clark, 1935; Blaisie, Goldman, Chacko & Dewey, 1972) have not described the changes in small-angle X-ray scattering which occur as humidity is altered.

Nerve bundles were dissected from the walking leg of the crab (*Carcinus maenas*) and placed in a humidity cell, where they remained unchanged in appearance during the 2–7 hr before exposure. Unstretched specimens were mounted vertically on a thin Melinex sheet in a humidity chamber in the hydrogen-filled X-ray camera. Humidity was altered after each exposure (duration ~ 1 hr).

At humidities above 80% there was no visible structure in the patterns

(Fig. 1). At 75 and 65% a single sharp ring was visible, equivalent to spacings of 53 and 50 Å respectively, but at 55% this was no longer apparent. At 44%, preferred orientation began to appear, and at 33% lamellar diffraction equivalent to a spacing of 61 Å was observed in the equatorial plane.

It is evident that changes in membrane stacking occurred with changes in humidity. The sharpness of the rings at 75 and 65% humidities suggests that the drying may cause lipid and protein to re-organize into a lamellar structure, but the tangled nature of the specimen prevents preferred orientation until lower humidities where shrinkage will have occurred.

Acknowledgments to Mr C. A. Wallace (GEC-Marconi, Borehamwood) for facilities and advice, to Dr P. R. Adams (St Bartholomew's Hospital Medical School, London) for specimens, to Drs A. E. Blaurock and D. Gilbert (King's College, London) for helpful discussion, and to the S.R.C. for a Research Studentship for G.R.C.

REFERENCES

BLAISIE, J. K., GOLDMAN, D. E., CHACKO, G. & DEWEY, M. (1972). *Biophys J.* **12**, 253a.

SCHMITT, F. O., BEAR, R. S. & CLARK, G. L. (1935). *Radiology* **25**, 131–151.

WORTHINGTON, C. R. (1971). *Biophysics and Physiology of Excitable Membranes*, ed. ADELMAN, W. J., pp. 1–46. Van Nostrand.

Slow recovery of the inactivation of sodium conductance in *Myxicola* giant axons

BY B. RUDY. *Physiological Laboratory, Cambridge CB2 3EG*

Although the sodium currents recorded under voltage-clamp conditions are basically similar in axons from squid and *Myxicola*, they exhibit a different frequency response in that squid axons can be pulsed at 10/sec or more for long periods with no change in the recorded currents, whereas in *Myxicola* even a rate of 1/sec results in a progressive decrease in the magnitude of the current until after some seconds a steady state is reached. This reduction in current size is not accompanied by any obvious change in time course. It takes place whether or not the potassium current is blocked with 4-aminopyridine (Fig. 1A), and for outward as well as inward currents (Fig. 1B).

The possibility has to be considered that this effect arises from depletion of the sodium in an external sink, or its accumulation in an internal sink. The double pulse experiment of Fig. 1A shows this to be unlikely, since the predicted alteration in the sodium equilibrium potential following the first pulse would tend to increase the current flowing outwards during the second pulse, and in fact a decrease is observed.

In the experiment of Fig. 1C, two identical test pulses were separated by

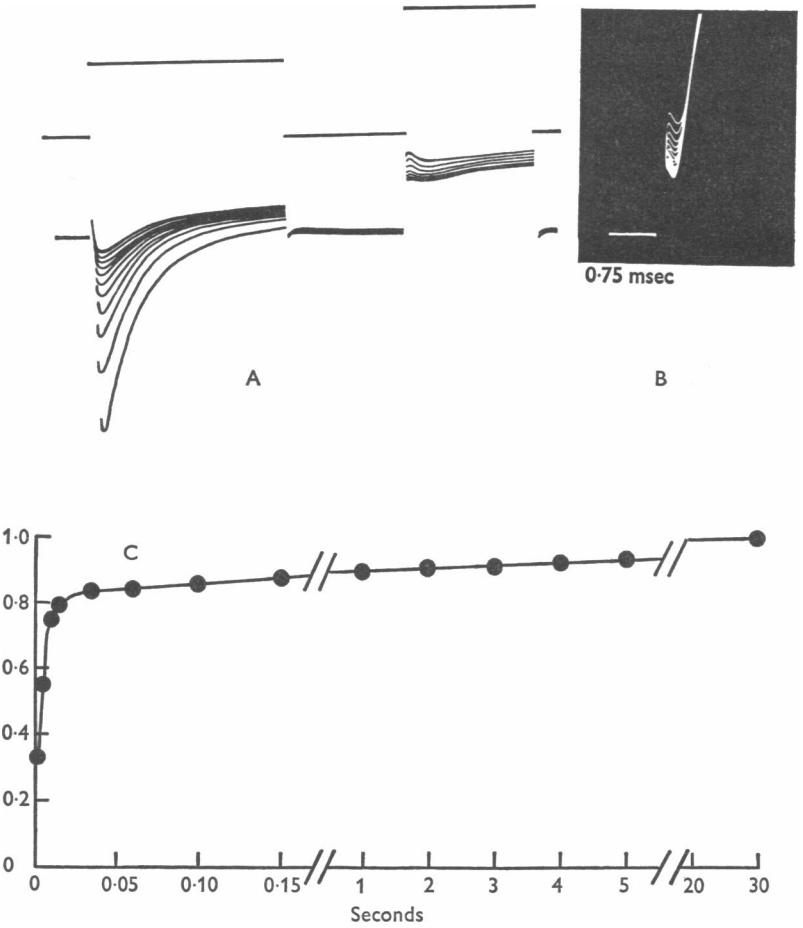

Fig. 1. Voltage-clamp records of membrane currents in axons from *Myxicola infundibulum* bathed in K-free artificial sea water (430 mM-NaCl, 10 mM-$CaCl_2$, 50 mM-$MgCl_2$, 10 mM-Tris Cl, pH 8). A, 13 superimposed sweeps. Holding potential −76 mV, first pulse to +24 mV for 6 msec, second pulse to +74 mV for 4 msec. Repetition rate 4/sec. Solution contained 5 mM 4-aminopyridine. Temperature 7° C. Compensated feed-back. Sodium equilibrium potential +54 mV. Peak current decreased from 0·9 to 0·07 mA/cm² during first pulse, and from 0·38 to 0·22 mA/cm² during the second one. B, 15 superimposed sweeps. Holding potential −86 mV, pulse to +84 mV. Repetition rate 4/sec. Temperature 10° C. Different axon from A. Peak outward sodium current decreased from 0·72 to 0·4 mA/cm². C, recovery from inactivation. Ratio of peak inward sodium current during test pulse to that during identical prepulse, plotted against time interval between the pulses. Holding potential −76 mV, pulses to +24 mV for 8 msec. Same axon as A, before the application of 4-aminopyridine.

the times indicated. An initial fast exponential recovery similar to that described for squid by Hodgkin & Huxley (1952) was followed by a much slower recovery phase that was only complete after about ½ min. In other experiments in which a test pulse followed a prepulse after an interval of 1 or 500 msec, the time constant for the onset of inactivation remained roughly constant, suggesting a single inactivation process with two phases of recovery rather than two independent processes with different time constants.

The support of the Overseas Development Ministry and of the Consejo Nacional de Ciencia y Tecnologia Mexico is gratefully acknowledged.

REFERENCE

HODGKIN, A. L. & HUXLEY, A. F. (1952). *J. Physiol.* **116**, 497–506.

The ionic permeability changes caused by the excitatory transmitter at the insect neuromuscular junction

BY R. ANWYL and P. N. R. USHERWOOD. *Department of Zoology, University of Nottingham, Nottingham NG7 2RD*

In a previous communication (Anwyl & Usherwood, 1974) we presented data on the ionic basis of the action of L-glutamate at the insect neuromuscular junction. We now have evidence from voltage-clamp studies that the action of the natural transmitter at this site is identical to that of L-glutamate, and that the excitatory post-synaptic current (e.p.s.c.) involves Na and K activation.

Fibres of the extensor tibiae muscle of the locust were voltage-clamped using a conventional 2-micro-electrode method. Inward currents recorded at excitatory synapses in response to neural stimulation, i.e. e.p.s.c.s, had a maximum amplitude of 5×10^{-6} A and a rise time of 4 msec at a membrane potential (E_m) of -55 to -60 mV. The e.p.s.c.s were progressively reduced in amplitude when E_m was lowered towards zero and they reversed in polarity at $+4$ mV.

In 10% Na saline (14 mM-Na), the amplitude of the e.p.s.c. was reduced by 90%, and the equilibrium potential of the e.p.s.c. (E_R) shifted to -8 mV. Changing external K between 0 and 20 mM (0–200% normal) did not alter E_R, but in 40 mM-K (400% normal), E_R shifted to $+12$ mV. Changing external Cl did not affect E_R.

Assuming that the excitatory transmitter causes a conductance increase to only Na and K then the ratio of the changes in sodium and potassium conductances $\Delta g\text{Na}/\Delta g\text{K}$ is 1·3 in normal saline. A change in E_R of 33 and 25 mV respectively would be expected for a tenfold change in external Na and K if $\Delta g\text{Na}/\Delta g\text{K}$ remained constant, as at the vertebrate end-plate

(Takeuchi & Takeuchi, 1960). Since the observed changes in E_R were much smaller than these predicted changes, it is possible that ΔgNa/ΔgK changes with the extracellular concentrations of these ions. Indeed, the observed changes in E_R are much closer to the theoretical values predicted by assuming that ΔpNa/ΔpK rather than ΔgNa/ΔgK remains constant during changes in extracellular K and Na. ΔpNa/ΔpK derived from the Goldman–Hodgkin–Katz (Goldman, 1943; Hodgkin & Katz, 1949) equation is 0·9 in normal saline. This equation predicts that E_R should change by 54 mV for a tenfold change in external Na but that a tenfold change in external K should result in a change in E_R of only 1·5 mV. At this insect excitatory nerve–muscle synapse changes in extracellular sodium produce shifts in E_R which are smaller than those predicted by assuming a constant ΔgNa/ΔgK, but this may be due to an increase in permeability to Ca at low external Na concentrations.

REFERENCES

ANWYL, R. & USHERWOOD, P. N. R. (1974). *J. Physiol.* **242**, 86–87*P*.
GOLDMAN, D. E. (1943). *J. gen. Physiol.* **27**, 37–60.
HODGKIN, A. L. & KATZ, B. (1949). *J. Physiol.* **108**, 37–77.
TAKEUCHI, A. & TAKEUCHI, N. (1960). *J. Physiol.* **154**, 52–67.

Changes in potassium activity within frog sartorius muscle fibres during sodium enrichment in potassium-free Ringer fluid

BY R. P. KERNAN and MARY MACDERMOTT. *Department of Physiology, University College, Earlsfort Terrace, Dublin 2, Ireland*

Armstrong & Lee (1971) using micro-electrodes made from ion selective glass (Eisenman, Rudin & Casby, 1957) measured activities of sodium and potassium ions in freshly isolated frog skeletal muscles and in their companion muscles which had been soaked in cold K-free Ringer during the night, in order to accumulate sodium. They found that the latter group of muscles lost an average of 26 m-equiv/l. fibre water of potassium in exchange for a similar quantity of sodium taken up, but that the activity of potassium in the fibres, a_{K_i}, decreased by only 8·4 m-equiv/l., with sodium activity increasing by as little as 4·7 m-equiv/l. under these conditions. They concluded that these cations were adsorbed or sequestered within the fibres.

We have measured changes in potassium equilibrium potentials, E_K, and of a_{K_i} in the fibres under similar conditions, by means of micro-electrodes containing liquid ion-exchanger, Corning 477317 (Walker, 1971) which shows a much greater specificity towards potassium with respect to sodium ions than does the glass electrode. Our electrodes were prepared by a modification of Walker's method in which 2% trimethylchlorosilane

in toluene was used to make hydrophobic the portion of the microcapillary tip which was to be filled subsequently by organic ion-exchanger. The potassium-selective micro-electrodes were calibrated against the standard 3 M-KCl-filled micro-electrodes in the following solutions; (*a*) 1, 10, 100 and 200 mM-KCl, (*b*) similar solutions in which Na^+ replaced K^+ as the concentration of the latter was reduced from 200 to 1 mM. In the former solutions the electrode potential changed by 55 mV at 15° for a decade change in a_{K_i}, in the latter the change was 53 mV.

Membrane potential, E_m, as well as E_K and a_{K_i} were measured in both sets of muscles during their immersion in normal Ringer. Following analysis of the wet ashed muscles by flame photometry, intracellular potassium concentrations, $[K]_i$ were calculated on the basis of inulin space and total muscle water. These values in freshly dissected muscles were $E_m - 92{\cdot}3 \pm 0{\cdot}1$ mV, $E_K - 100{\cdot}3 \pm 0{\cdot}2$ mV, a_{K_i} $91{\cdot}2 \pm 1{\cdot}4$ m-equiv/l., $[K]_i$ $128 \pm 1{\cdot}5$ m-equiv/l. and in sodium-enriched muscles, $E_m - 78{\cdot}9 \pm 0{\cdot}7$ mV, $E_K - 76{\cdot}7 \pm 0{\cdot}8$ mV, a_{K_i} $67{\cdot}8 \pm 1{\cdot}2$ m-equiv/l., $[K]_i$ $80{\cdot}6 \pm 2{\cdot}7$ m-equiv/l.

It is evident from these results that while the fibres lost about 48 m-equiv/l. of potassium in K-free ringer, a_{K_i} decreased by only 23·4 m-equiv/l. Assuming an activity coefficient, γ_{K_i} of about 0·7 for intracellular potassium the expected fall would be 33·6 m-equiv/l. The discrepancy here which is much less than that found by Armstrong & Lee could be accounted for if γ_{K_i} increased from 0·70 to 0·83 during potassium loss. The mean measured E_K value of Na-rich muscles, $-76{\cdot}7$ mV, was significantly less than the value of 84·5 mV calculated from concentrations alone.

This work was supported by a grant from the Medical Research Council of Ireland.

REFERENCES

ARMSTRONG, W. McD. & LEE, C. O. (1971). *Science, N.Y.* **171**, 413–415.
EISENMAN, E., RUDIN, D. O. & CASBY, J. V. (1957). *Science, N.Y.* **126**, 831–834.
WALKER, J. L. (1971). *Analyt. Chem.* **43**, 89A–92A.

Relations between membrane potential, sodium currents and contraction in frog twitch muscle fibres

BY JACQUES CAILLÉ, MICHÈLE ILDEFONSE and OGER ROUGIER. *Laboratoire de Physiologie des Elements Excitables, Université Claude Bernard, 43 Boulevard du 11 Novembre 1918, 69621, Villeurbanne, France*

There is evidence for a Na-dependent action potential in the T system (Costantin, 1970; Bezanilla, Caputo, Gonzales-Serrato & Venosa, 1972; Bastian & Nakajima, 1974), which may prevent clamping of the tubular membrane at the fibre centre (Adrian & Peachey, 1973). To obtain further

information on this problem we have recorded initial current and contraction using a double sucrose-gap apparatus.

In Ringer, for depolarizations between 2 and 3 msec, the mechanical threshold is near the sodium threshold. The amplitude of the contraction increases progressively with further depolarization towards a maximum value, without any modification of its time to peak.

When the test pulse lies below E_{Na}, a conditioning depolarization inactivates both sodium current and contraction, without modification of the time to peak of contraction. Conditioning has no effect when the test pulse is near E_{Na}.

In tetrodotoxin Ringer (10^{-7}) the mechanical threshold is 10–30 mV more positive. For depolarizations below E_{Na}, sodium current and contraction are decreased proportionately; the time to peak of contraction is not modified. Near the sodium equilibrium potential, the contraction is not altered by tetrodotoxin.

The reversibility of the tetrodotoxin action is never complete but the sodium current returns towards control values in a few minutes, while the contraction needs several tens of minutes to recover.

When the tetrodotoxin concentration is reduced to 10^{-9}, its action on the initial current and on the contraction develops in two phases: at first, the peak of inward current is reduced without any decrease of the contraction. Later, tetrodotoxin acts on a second slower phase of the initial current and the contraction is reduced without modification of its time to peak.

Such an action of tetrodotoxin (10^{-9}) is also observed in current-clamp experiments, where the spike of the action potential decreases first without any modification of the contraction. Then, a slower action potential develops and decreases together with a decrease of the contraction. In this case, the time to peak of contraction is increased.

These experiments show again that a part of contraction is dependent on a regenerative sodium process in the tubular membrane. A very weak tetrodotoxin concentration allows the separation of two components of the sodium current or of the action potential, which may concern respectively the surface membrane and the tubular membrane.

From Adrian & Peachey's (1973) calculations, the action of tetrodotoxin and of pre-depolarizations on contractions might be explained by the inhibition of the tubular action potential. The contraction obtained for depolarizations near the sodium equilibrium potential should then be altered by tetrodotoxin or pre-depolarizations. Moreover, whenever the contraction is altered, a modification of its time to peak should be observed. These effects do not occur in our experimental conditions.

It is therefore possible that the tubular membrane may be better

clamped than predicted by Adrian & Peachey. In this case, a direct relation could be envisaged between the tubular sodium current and a part of contraction.

REFERENCES

ADRIAN, R. H. & PEACHEY, L. D. (1973). *J. Physiol.* **235**, 103–131.
BASTIAN, J. & NAKAJIMA, S. (1974). *J. gen. Physiol.* **63**, 257–278.
BEZANILLA, F., CAPUTO, C., GONZALES-SERRATOS, H. & VENOSA, R. A. (1972). *J. Physiol.* **223**, 507–523.
COSTANTIN, L. L. (1970). *J. gen. Physiol.* **55**, 703–715.

The sphincter pupillae: contractions in Ca^{2+}-free solution

BY G. GABELLA and L. RAEYMAEKERS. *Department of Anatomy, University College London, Gower St, London WC1 6BT*

Smooth muscles differ in their ability to contract in the absence of extracellular Ca^{2+}. The aorta and pulmonary artery still develop about 25% of their maximal tension in response to noradrenaline after 30 min in a Ca^{2+}-free medium, whereas under the same conditions, the contractions of the mesenteric vein, and those of the taenia coli in response to acetylcholine, are abolished (Devine, Somlyo & Somlyo, 1972). In the guinea-pig taenia coli we observed that in Ca^{2+}-free medium contractions in response to carbachol declined very rapidly and disappeared within 3 min. Experiments were carried out on the guinea-pig iris. The two eyes, from which the cornea had been excised, were superfused *in vitro* with normal Krebs solution without phosphate. Photographs of the iris were taken throughout the experiments with the aid of a stereomicroscope. In normal Krebs solution control contractions of the sphincter pupillae, obtained by applying carbachol (5×10^{-6} M), were sustained; they reduced the pupil diameter from 4 to about 1·5 mm. K^+-depolarization caused a constriction which was slightly smaller than that of carbachol and receded after about 5 sec, possibly because both the sphincter and the dilator muscles were activated. When the extracellular Ca^{2+} of the sphincter pupillae was depleted by superfusion with a Ca^{2+}-free Krebs solution containing 2 mM-EGTA, K^+ contractions no longer occurred and the carbachol-induced contractions became not sustained and declined in amplitude and duration with time of exposure. However, whereas the K^+ contractions were abolished within 1 min or less, the muscle still contracted in response to carbachol to about 50% of the maximal response after 3 min in Ca^{2+}-free medium, a time at which no contraction can be elicited in the taenia coli. The contractions declined to about 20% after 15 min. The effect of a Ca^{2+}-free solution on the K^+ contractions indicates that depletion of extracellular Ca^{2+} occurs rapidly, which is accounted for by the fact that the muscle is only about 40 μm thick. Carbachol contrac-

tions in Ca^{2+}-free solution suggest that this muscle has an intracellular store of calcium, which allows it to maintain the ability to contract for a long time. It is known that the muscle cells of the sphincter pupillae differ from those of the taenia coli in at least three respects – higher surface-to-volume ratio, greater number of mitochondria, and more highly developed sarcoplasmic reticulum (Gabella, 1974). It is, however, not known where the intracellular calcium is stored.

Supported by the Wellcome Trust.

REFERENCES

DEVINE, C. E., SOMLYO, A. V. & SOMLYO, A. P. (1972). *J. cell Biol.* **52**, 690–718.
GABELLA, G. (1974). *Proc. R. Soc.* B **186**, 369–386.

Rectification in the smooth-muscle cell membrane of the rabbit aorta

BY F. MEKATA. *Department of Physiology, London Hospital Medical College, Turner Street, London E1 2AD*

Mammalian smooth muscles have sarcoplasmic reticulum which in general does not appear to communicate with the cell membrane. The sarcoplasmic reticulum might therefore have little influence on the electrical properties of the smooth muscle cell membrane. The current–voltage relation of rabbit aorta has now been measured by the partitioned chamber method (Mekata, 1971, 1974). In both normal Krebs and Na-free choline solutions containing 5·9 mM-$[K]_o$, strong cathodal rectification and clear delayed rectification in the outward-going direction were observed. In both Na and Na-free choline solutions containing 96 mM-$[K]_o$, rectification of the aorta was weakened, but anomalous rectification was never observed. Decrease in slow potassium conductance during application of large and long-lasting hyperpolarizing current, which Adrian & Freygang (1962) found in skeletal muscle, was never observed in the solutions containing 5·9 mM-$[K]_o$. It is suggested from the present experiments that the absence of this and of anomalous rectification in the aortic muscle is associated with the absence of sarcoplasmic reticulum communicating with the surface membrane.

REFERENCES

ADRIAN, R. H. & FREYGANG, W. H. (1962). *J. Physiol.* **163**, 61–103.
MEKATA, F. (1971). *J. gen. Physiol.* **57**, 738–751.
MEKATA, F. (1974). *J. Physiol.* **242**, 143–155.

Evidence concerning the site of receptors mediating the Hering–Breuer inflation reflex

By Lucille Bitensky, D. J. Chambers, J. Chayen, Brenda A. Cross, A. Guz, S. K. Jain and J. J. Johnstone. *Division of Cellular Biology, Kennedy Institute and Department of Medicine, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF*

Pulmonary stretch receptors have been thought to be located in airway smooth muscle (Widdicombe, 1964). Cholinergic parasympathetic motor endings are also distributed to airway smooth muscle (Fillenz & Widdicombe, 1972). Inhalation of an aerosol of aqueous 5% bupivacaine hydrochloride has been shown in the rabbit to block the cough, inflation and deflation reflexes while preserving parasympathetic bronchomotor function and the ventilatory response to 'J'-receptor stimulation with phenyldiguanide (Jain, Trenchard, Reynolds, Noble & Guz, 1973). The diameter of aerosol particles (5–19 μm) could explain the failure to block receptors at alveolar level. However, the inability to block parasympathetic motor endings was surprising and led to an investigation of the exact location of stretch receptors which had been blocked. This was studied with autoradiography using tritiated bupivacaine (1-butyl-2′,6′ pipecoloxylidide [pipecolyl-^{3}H]) of 99% purity assessed by thin-layer radiochromatography. Four ml. 5% bupivacaine hydrochloride solution containing 2 mCi/ml. were aerosolized and administered with positive pressure inflations to an anaesthetized rabbit, until the apnoeic response to lung inflation was abolished (Jain *et al.* 1973).

Cryostat sections of the lung were prepared and examined by a dry autoradiographic technique (Appleton, 1964). Results showed that: (1) the label was distributed throughout the airway, down to bronchioles of 200 μm diameter; (2) the label was predominantly concentrated in the epithelium, but had penetrated the basement membrane to reach the lamina propria superficial to the smooth muscle mass (Fig. 1); (3) little label could be seen in alveolar walls. The relative failure of bupivacaine to penetrate the bronchial smooth-muscle mass at a time when the inflation reflex was blocked accords with the preservation of parasympathetic motor function. It also strongly supports the recent suggestion by von During, Andres & Iravani (1974) that the endings of pulmonary stretch fibres may lie just beneath the basement membrane.

The Charing Cross Research Subcommittee is thanked for the purchase of the titrated bupivacaine and the Wellcome Trust for a personal grant to S.K.J.

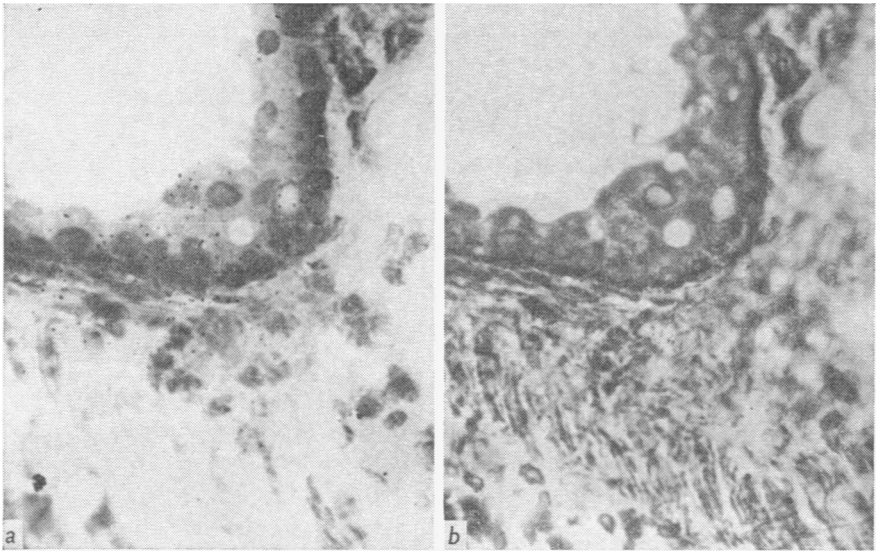

Fig. 1. Haematoxylin-eosin stained section with autoradiograph of a bronchiole (200 μm) (*a*) by normal illumination to show the distribution of dark silver grains, (*b*) by phase-contrast to show the basement membrane (dark line at base of epithelium). (× 1200.)

REFERENCES

APPLETON, T. C. (1964). *Jl R. microsc. Soc.* **83**, 277.

FILLENZ, M. & WIDDICOMBE, J. G. (1972). In *Enteroreceptors*, ed. NEIL, E., pp. 81–112. Berlin: Springer.

JAIN, S. K., TRENCHARD, D., REYNOLDS, F., NOBLE, M. I. M. & GUZ, A. (1973). *Clin. Sci.* **240**, 91–109.

VON DURING, M., ANDRES, K. H. & IRAVANI, J. (1974). *Z. Anat. EntwGesch.* **143**, 215–222.

WIDDICOMBE, J. G. (1964). In *Handbook of Physiology*, vol. I, ed. FENN, W. O. & RAHN, H., pp. 585–630. Washington, D.C.: American Physiological Society.

Effects of histamine, 2-methylhistamine and 4-methylhistamine on blood pressure and vascular resistance in the cat

BY R. W. BRIMBLECOMBE, SHEILA B. FLYNN and D. A. A. OWEN.* *The Research Institute, Smith Kline and French Laboratories Ltd, Welwyn Garden City, Hertfordshire*

Two receptor populations, defined as H_1- (Ash & Schild, 1966) and H_2-receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972) have been described for histamine.

Both types of receptors are involved in the depressor and vasodilator

responses to histamine in the cat (Owen & Parsons, 1974; Flynn & Owen, 1974). Selective agonists have also been described for histamine receptors; 2-methylhistamine shows relative selectivity for H_1-receptors whereas 4-methylhistamine has relative selectivity for H_2-receptors (Black *et al.* 1972).

We have investigated the depressor and vasodilator activity of 2-methylhistamine and 4-methylhistamine and compared their effects with those of histamine.

Experiments have been made in cats anaesthetized with sodium pentobarbitone, 60 mg/kg I.P. The effects of intravenous injections of histamine, 2-methylhistamine and 4-methylhistamine were measured on blood pressure, recorded from the right common carotid artery. The vasodilator activity of the compounds was studied in the acutely denervated left hind limb perfused with blood at constant flow. The vasodilator responses to intra-arterial injections were measured as falls in perfusion pressure.

Each of the compounds tested elicited dose-dependent falls in both blood pressure and hind-limb perfusion pressure and the findings on both preparations were similar.

H_1-receptor blockade with mepyramine caused displacement to the right of the histamine dose–response curve. This reached a maximum and further displacement required H_2-receptor blockade with metiamide. Mepyramine also caused displacement to the right of the 2-methylhistamine dose–response curve. The maximum displacement of the 2-methylhistamine dose–response curve by mepyramine exceeded the maximum displacement of the histamine dose–response curve.

In contrast, metiamide alone, which did not change histamine or 2-methylhistamine responses, caused displacement of the 4-methylhistamine dose–response curve. This displacement was not very large and further displacement could only be achieved by administration of mepyramine.

These experiments confirm the involvement of both H_1- and H_2-receptors in depressor and vasodilator responses to histamine. Both 2-methylhistamine and 4-methylhistamine are of value to determine the receptors involved in histamine-mediated responses. Caution should, however, be used in interpretation of the results when large doses of these agonists are used since their specificity is not absolute.

REFERENCES

ASH, A. S. F. & SCHILD, H. O. (1966). *Br. J. Pharmac.* **27**, 427–439.

BLACK, J. W., DUNCAN, W. A. M., DURANT, G. J., GANELLIN, C. R. & PARSONS, M. E. (1972). *Nature, Lond.* **236**, 385–390.

FLYNN, S. B. & OWEN, D. A. A. (1974). *Br. J. Pharmac.* **52**, 122P.

OWEN, D. A. A. & PARSONS, M. E. (1974). *Br. J. Pharmac.* **51**, 123–124P.

The effect of warming and cooling the hypothalamus, or intracarotid injections of cold saline, on thermoregulatory behaviour in sheep

By B. A. Baldwin and J. O. Yates. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

Six shorn sheep, placed in a temperature-controlled room held at 5° C, were trained to press a panel switch with their muzzles in order to obtain heat from a 2·7 kW array of infra-red (I.R.) lamps. The sheep were loosely restrained in a metal stand by means of a leather harness and each press on the panel resulted in the delivery of 7 sec of I.R. heat.

The sheep were implanted, in the mid line of the preoptic region of the hypothalamus, with thermodes through which cold alcohol or warm water could be circulated (Baldwin & Ingram, 1967). Surface temperature on the thermode was monitored using a thermistor, which was also used to record hypothalamic temperature when the thermode was not in use. Three of the sheep were provided with bilateral carotid loops into which catheters were inserted to facilitate intracarotid injections by means of a peristaltic pump.

Cooling the hypothalamus. Four sheep were exposed individually to ambient temperatures of 5, 15, 25 and 35° C for periods of 140 min. During this time there were three 20 min periods in which the thermode was cooled to 15° C. Each sheep was exposed to each ambient temperature on three occasions. The periods of cooling the hypothalamus produced, at all ambient temperatures, a marked acceleration in the rate at which the heaters were used. At 25 and 35° C the peak rates seen during cooling were considerably below those seen at 5 and 15° C.

Warming the hypothalamus. Three sheep were exposed individually to ambient temperatures of 5 and 15° C for 140 min periods and during this time there occurred three 20 min periods in which the thermode was warmed to 43° C. Each sheep was exposed three times to each ambient temperature and during all the warming episodes there was a marked reduction in the rate of using the I.R. heaters.

Intracarotid injections. In three sheep intracarotid injections of cold (about 3 °C) normal saline were made as they operated the heaters at ambient temperatures of 15, 25 and 35° C. During an experiment lasting 2 hr, two injections, each of which lasted 15 min, were made. The sheep showed no signs of discomfort during the injections. Each sheep was tested six times at each ambient temperature and at 15, 25 and 35° C the injections, which lowered hypothalamic temperature by 1° C, invariably resulted in a marked increase in the rate of using the heaters. During the injections deep body temperature fell by about 0·1° C. At 15° C it was shown that lowering hypothalamic temperature by 0·6° C also increased the use of the heaters.

REFERENCE

BALDWIN, B. A. & INGRAM, D. L. (1967). *J. Physiol.* **191**, 375–392.

Skin temperatures and hyperventilation during cold water immersion

BY K. E. COOPER and SHEILAGH M. MARTIN. *Division of Medical Physiology, University of Calgary, Calgary, Alberta, Canada*

Cyclic adenosine 3′-5′-monophosphate (cAMP) and body temperature

BY M. J. DASCOMBE and A. S. MILTON. *Department of Pharmacology, University of Aberdeen, Aberdeen AB9 2ZD, Scotland*

Cyclic adenosine 3′-5′-monophosphate (cAMP), its dibutyryl derivative (Db-cAMP) and ATP, ADP and AMP have been injected into the region of the anterior hypothalamus of the unanaesthetized cat, and both the long- and short-term effects of these drugs on body temperature recorded. In addition, the autonomic and behavioural effects following the injection of these substances have been observed. Db-cAMP and cAMP both produced hypothermia when applied to the preoptic–anterior hypothalamus (PO/AH). With Db-cAMP, the hypothermia was shown to be dose-dependent between 50 and 500 μg (0·096–0·96 μmol). AMP, ADP and ATP also produced hypothermia when injected into the PO/AH. The order of relative potencies of the adenine nucleotides with respect both to the hypothermia produced and to the autonomic thermoregulatory effects observed were similar. Db-cAMP was most potent and cAMP least. In some animals the hypothermic response to the adenine derivatives was followed by a long-lasting rise in deep body temperature. Indeed, micro-injection into the PO/AH of many substances including saline produced in most cats a non-specific rise in body temperature apparently the result of tissue damage. Intraperitoneal injection of 4-acetamidophenol (paracetamol 50 mg/kg) reduced or abolished this febrile response. The hypothermic effect of the adenine nucleotides have been compared with the effects produced in these same cats by micro-injections of noradrenaline, 5-hydroxytryptamine, a mixture of acetylcholine and physostigmine (1:1), EDTA and excess Ca^{2+} ions. It is concluded that as Db-cAMP and cAMP both produce hypothermia, it is unlikely that endogenous cAMP in the PO/AH mediates the hyperthermic responses to pyrogens and prostaglandins.

M. J. Dascombe is in receipt of a Medical Research Council scholarship.
The research was supported by a grant from the Medical Research Council.

Modulation by oestrogen of the rate of precocious sexual maturation in female rats induced by brain stimulation

BY J. H. KITCHEN,* K. B. RUF and M. WILKINSON. *Department of Physiologie, University of Geneva, Switzerland*

In a previous study (Ruf, YoungLai & Holmes, 1974) electrochemical stimulation of the basal hypothalamus was found to be an efficient technique for the induction of precocious sexual maturation in immature female rats. The concentration of ovarian oestrogen was elevated as early as 1 hr after brain stimulation, and oestrogen production was increased over subsequent days as judged by the extent of uterine hypertrophy. Tubal ova were present 96 or 120 hr after brain stimulation in about 50% of these rats. Since precocious puberty can also be induced by repeated administration of oestrogen (Ramirez & Sawyer, 1965), we elected to study the possible interaction between brain stimulation and exogenous oestradiol.

23-day-old rats of a Sprague–Dawley strain, weighing $\geqq$ 56 g, were injected s.c. with a single dose of 0·25 μg oestradiol benzoate (OB) immediately after unilateral stimulation of the arcuate–ventromedial area through a stainless-steel electrode (0·5 mA d.c., 15 sec). In 6 out of 40 rats, a full set of tubal ova was present on day 26, i.e. 72 hr after brain stimulation. When no steroid was administered, such an early ovulatory response was neither observed in this series nor in 260 rats previously subjected to brain stimulation (Ruf *et al.* 1974). A similar proportion (5 out of 33 rats) of early ovulators was observed when the priming dose of OB was increased to 0·5 μg/rat. With both doses of OB the peak ovulatory response occurred on day 27 as with brain stimulation alone, but was delayed in a considerable number of OB-primed animals. This indicates that positive and negative feed-back effects of the steroid may be superimposed and that an oestrogen-primed rat may fall into either category depending on the rate of endogenous steroid production induced by the brain stimulus. Neither dose of OB changed the occurrence of first spontaneous ovulation when administered alone.

Radio-immunological determinations of gonadotropins in anterior pituitary and in plasma indicate that both OB-priming and brain stimulation lead to a prompt and sustained increase in the LH/FSH ratio. The observed shift in pituitary gonadotropin output extends the early observations of Hohlweg (1936) on positive feed-back effects of oestrogen during pubertal development and suggests that brain stimulation advances sexual maturation primarily by activation of this feed-back loop.

* Present address: Department of Physiology, University of Texas, Southwestern Medical School, Dallas, Texas 75235 U.S.A.

This research is supported by grant no. 3.016.73 from the Swiss National Science Foundation. The receipt of NIAMDD radio-immunoassay reagents is gratefully acknowledged.

REFERENCES

HOHLWEG, H. (1936). *Klin. Wschr.* **15**, 1832–1835.
RAMIREZ, V. D. & SAWYER, C. H. (1965). *Endocrinology* **76**, 1158–1168.
RUF, K. B., YOUNGLAI, E. V. & HOLMES, M. J. (1974). *Brain Res.* **78**, 437–446.

Variation in plasma ACTH levels during the human menstrual cycle

BY J. K. BURNS. *Department of Physiology, University College, Galway*

It was previously proposed that a neuroendocrine cycle may explain the profound and unique increase in urinary oestrediol output during human pregnancy (Burns, 1971). This cycle involves stress and oestrogenic stimulation of pituitary secretion of ACTH. Increase in maternal and foetal pituitary hormones are considered to stimulate adrenocortical formation of dehydroepiandrosterone sulphate (DHAS). Hydroxylation of DHAS by the foetal liver is followed by its conversion by the placenta into oestrogens. These are considered to increase ACTH production. This proposal, of a neuroendocrine cycle, has been supported by a recent observation of increased ACTH levels during human pregnancy (Burns, 1975).

The present investigation involved measurement of plasma ACTH levels during the menstrual cycle using a radio-immunoassay technique (Croughs, Tops & de Jong, 1973). Plasma levels of ACTH were measured during seven different phases of the menstrual cycle in ten women. These phases were of 4 days duration each and coincided with menstrual, early and late proliferative, mid-cycle, early, intermediate and late secretory. Levels of ACTH at various phases of the cycle were compared with those for the menstrual phase, when the value for plasma ACTH was $83{\cdot}3 \pm 9{\cdot}8$ pg/ml. Mid-cycle levels only were significantly different ($165{\cdot}0 \pm 14{\cdot}4$ pg/ml.) and the increase in concentration was highly significant ($P < 0{\cdot}01$). This finding appears to support stimulation by oestrogens of human pituitary ACTH production.

REFERENCES

BURNS, J. K. (1971). In *Psychosomatic Medicine in Obstetrics and Gynaecology*, ed. MORRIS, N., pp. 222–225. Basel: Karger.
BURNS, J. K. (1975). *J. Physiol.* **246**, 106–107*P*.
CROUGHS, R. J. M., TOPS, C. F. & DE JONG, F. H. (1973). *J. Endocr.* **59**, 439–449.

Calcium uptake by axoplasm extruded from giant axons of *Loligo*

By P. F. Baker and W. Schlaepfer. *Department of Physiology, King's College, Strand, London, and The Laboratory of the Marine Biological Association, Plymouth*

Squid axoplasm contains about 500 μmole/kg of calcium, the bulk of which is bound. Very little Ca is free to move in an electric field and experiments with aequorin have shown that the concentration of ionized Ca is less than 1 μM (Baker, 1972). The sequestration of Ca by mitochondria is well established and the observation that exposure of axons to mitochondrial inhibitors results in a rise in axoplasmic ionized Ca of 10- to 100-fold is fully consistent with a role for mitochondria in axoplasmic Ca-binding.

The calcium-binding systems persist for many hours in extruded axoplasm. This can be shown by extruding axoplasm into a tube of diameter roughly equal to that of the axon. Injection of aequorin reveals an ionized Ca concentration close to that in intact axons and subsequent injection of enough Ca to raise the calcium content by 60 μmole/kg causes only a transient rise in ionized calcium.

In order to characterize these Ca-binding systems more fully we have measured the uptake of ^{45}Ca into axoplasm held within small dialysis sacs and immersed in a solution of composition: KCl, 350 mM; sucrose, 500 mM; $MgCl_2$, 10 mM; sodium succinate, 10 mM; sodium phosphate, 10 mM; ATP, 5 mM; $CaCl_2$, 5–10 μM; Tris buffer, pH 7·2, 10 mM. Accumulation ratios (^{45}Ca axoplasm/^{45}Ca medium) often exceeded thirty. Removal of ATP, P_i and succinate and addition of cyanide (2 mM) and oligomycin (5 μg/ml.) reduced but failed to abolish accumulation and provided a convenient means of separating two Ca-binding components: a component that requires either ATP and P_i or succinate and P_i, that is sensitive to cyanide and oligomycin and is identical in properties to a crude preparation of mitrochondria isolated from axoplasm and a second component that persists under conditions where mitochondrial uptake has been abolished and probably reflects binding of Ca to axoplasmic protein.

Other properties of these two components are also quite different. Mitochondrial calcium uptake is half maximal in the Ca concentration range 20–40 μM and uptake continues for many hours. The presumed non-mitochondrial binding system equilibrates rapidly with the available Ca and it is possible to distinguish a high affinity component of binding that is half-maximal at about 0·5 μM-Ca (capacity 20–40 μmole Ca^{2+}/kg axoplasm) and a low affinity component that is not saturated at 500 μM-Ca.

Two particularly interesting aspects of these experiments are: (1) axoplasmic uptake of Ca is dependent on the monovalent cation composition of the medium, being highest in K and lower in Na, Li or Tris and

(2) uptake is reduced by heavy metals such as La^{3+} and Mn^{2+}. The mitochondria seem most sensitive to the monovalent cation composition of the medium whereas the non-mitochondrial binding system seems most sensitive to lanthanum.

REFERENCE

BAKER, P. F. (1972). *Prog. Biophys. molec. Biol.* **24**, 177–223.

Hormone release in relation to inactivation of Ca-entry in the rat neurohypophysis

BY J. J. NORDMANN. *Department of Pharmacology, University of Cambridge, Cambridge CB2 2QD*

Incubation of isolated rat neurohypophyses in the presence of a raised $[K]_o$ elicits the release of oxytocin and vasopressin (Douglas & Poisner, 1964*a*). Thus, exposure to a potassium concentration of 56 mM (i.e. a tenfold increase in $[K]_o$) for 10 min causes a transient increase in hormone output, but, despite the brief exposure to a raised $[K]_o$, recovery of the output to the basal level is prolonged and takes approximately 120 min; the K-induced hormone output is dependent upon the presence of external calcium, and an uptake of ^{45}Ca is associated with evoked hormone release (Douglas & Poisner, 1964*b*). On the other hand when the neurohypophysis is incubated in $[K]_o$, 56 mM, for a more extended time period, i.e. 120 min, the hormone output is still not maintained and the time course of release and the total amount of hormone secreted remains indistinguishable from that observed after only 10 min exposure to a raised $[K]_o$.

These results might be explained either by rapid depletion of a releasable pool of neurohypophyseal hormone or by an inactivation of the secretory mechanism. The first alternative seems a doubtful possibility since the amount of hormone released by $[K]_o$, 56 mM, in a Na-free solution was found to be 5 or 6 times greater than that liberated in the presence of NaCl, 100 mM (Dreifuss, Grau & Bianchi, 1971), yet the time course of secretion was similar in both cases. The second alternative seems the more likely and could be closely related to inactivation of Ca-entry into the nerve terminal, for Fig. 1 shows clearly that the uptake of ^{45}Ca into the neurohypophysis on continuous exposure to $[K]_o$, 56 mM, decreases in an almost identical manner to hormone release.

These findings therefore indicate that the inability of the neurohypophysis to maintain a high secretory rate in the presence of a raised $[K]_o$ could be explained by inactivation of Ca-entry (see Baker, Meves & Ridgway, 1973; Baker & Rink, 1974). This may or may not be associated with the failure of an increase $[K]_o$ to maintain nerve terminal depolarization.

J.J.N. is a European Exchange Programme Research Fellow.

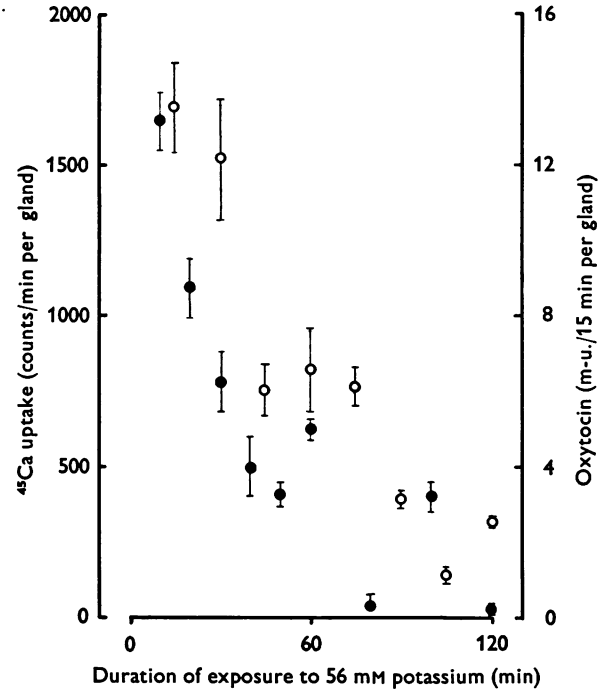

Fig. 1. Effect of a high $[K]_o$ on hormone release and ^{45}Ca uptake by isolated rat neurohypophyses. After pre-incubation for 15 min in a solution containing: $KHCO_3$, 5·6 mM; NaCl, 100 mM; $CaCl_2$, 2·2 mM; $MgCl_2$, 1·0 mM; choline chloride, 50 mM and glucose, 10 mM, the glands were transferred to a solution in which choline chloride has been replaced isosmotically by potassium chloride (50·4 mM). The solution was removed every 15 min and replaced by fresh medium. Each open circle (○) represents the mean oxytocin content (± S.E. of mean, $n = 6$) of the incubation medium for each time period. Oxytocin was estimated in a rat milk-ejection bio-assay. The ^{45}Ca uptake was determined in parallel experiments by adding ^{45}Ca, 10 μCi/ml., to the medium during the last 10 min of exposure to potassium, 56 mM. Prior to measurements of ^{45}Ca uptake the glands were washed for 60 min in fresh Locke solution in which the sodium was replaced by choline. Each filled circle (●) represents the mean stimulated ^{45}Ca uptake (S.E. of mean, $4 \leqslant n \leqslant 11$) after subtraction of the basal value obtained in control experiments (5·6 mM-KCl).

REFERENCES

BAKER, P. F., MEVES, H. & RIDGWAY, E. B. (1973). *J. Physiol.* **231**, 527–548.
BAKER, P. F. & RINK, T. J. (1974). *J. Physiol.* **241**, 107–109*P*.
DOUGLAS, W. W. & POISNER, A. M. (1964*a*). *J. Physiol.* **172**, 1–18.
DOUGLAS, W. W. & POISNER, A. M. (1964*b*). *J. Physiol.* **172**, 19–30.
DREIFUSS, J. J., GRAU, J. D. & BIANCHI, R. E. (1971). *Experientia* **27**, 1295–1296.

Transient inward current underlying strophanthidin's enhancement of pace-maker activity in Purkinje fibres

By W. J. Lederer and R. W. Tsien.* *Department of Physiology, Yale University School of Medicine, New Haven, Connecticut, U.S.A. 06510*

During digitalis toxicity, spontaneous impulses may arise in the ventricles as a result of enhanced pace-maker activity in the specialized conducting system. The cellular basis of such automaticity has been studied in isolated Purkinje fibres, and Ferrier & Moe (1973) have suggested that cardiotonic steroids produce transient depolarizations by inducing a transient Ca^{2+} influx. If their hypothesis is correct, diastolic depolarization under the influence of cardiotonic agents would differ from normal pacemaker activity in its ionic basis.

We have tested this explanation by studying the influence of strophanthidin on membrane currents in voltage-clamp experiments (Fig. 1). In (A), a train of action potentials was followed by activating the voltage clamp at the previous maximum diastolic potential (MDP). The ensuing current change reflects the slow decay of outward i_{K_2}, which normally controls the pacemaker depolarization.

Exposure to strophanthidin (B) produced a less negative MDP and increased the diastolic depolarization. The magnitude of the diastolic depolarization grew beat by beat, as previously reported (e.g. Davis, 1973). Clamping the membrane potential at MDP then gave a transient inward current (TI), superimposed on some decay of i_{K_2}. A similar TI appeared following a depolarizing clamp pulse. The magnitude and time course of the TI were appropriate to account for the observed increase in diastolic depolarization.

In other experiments, intracellular recording with a third micro-electrode showed good longitudinal uniformity during the TI. The amplitude of the TI rose with increases in strength or duration of the preceding depolarization, in accord with the enhancement of diastolic depolarization by repetitive stimulation (B). Removal of $[Ca]_o$ rapidly and reversibly abolished the TI, supporting the idea that Ca^{2+} carries the transient current. However, the kinetics of the TI are quite different to the normal Ca^{2+} pathway, the slow inward current. Replacement of $[Na]_o$ by choline increased the TI amplitude more than twofold, suggesting that Na^+ ions do not carry the transient current. It seems possible that either strophanthidin or $[Na]_o$ removal elevate $[Ca]_i$ (see Baker, 1972) and that $[Ca]_i$ somehow influences the TI. A rise in $[Ca]_i$ might be a common factor in the inotropic and arrhythmogenic actions of digitalis.

* Established Investigator of the American Heart Association.

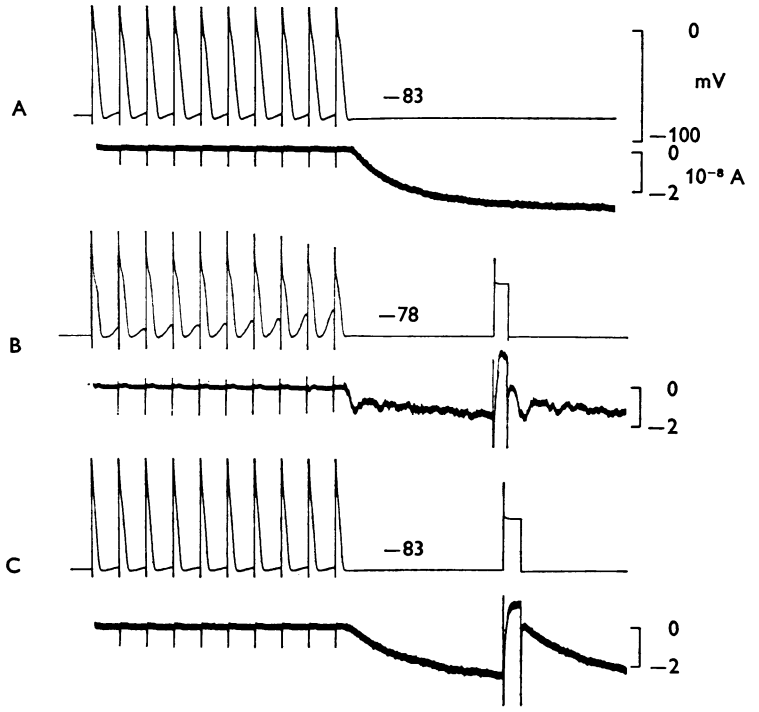

Fig. 1. Effect of strophanthidin on electrical activity and membrane current over the pace-maker range of potentials. A shortened calf Purkinje fibre preparation was impaled with two micro-electrodes and voltage clamped using the method of Deck, Kern & Trautwein (1964). Each panel shows chart recordings of membrane potential (above) and total membrane current (below). A train of action potentials was stimulated by external shocks at 0·5 Hz after a rest period of 25 sec. Voltage-clamp control was imposed following the tenth action potential at the point of maximum repolarization. This protocol allows a direct comparison between the chronotropic effects of strophanthidin and adrenaline (see Tsien, 1974, Fig. 2). (A) control run, (B) after exposure to 1 μM strophantidin for 27 min, (C) after washing off the strophanthidin for 25 min. Depolarizing voltage pulses lasting about 1 sec were applied in (B) and (C) and show respectively the occurrence of the TI phenomenon, and its disappearance after removal of the drug. The increase in current 'noise' in (B) is also reversible. Preparation 127–2, 35° C, $[Ca]_o$ = 5·4 mM, total capacitance = 0·063 μF.

REFERENCES

Baker, P. F. (1972). *Prog. Biophys. molec. Biol.* **24**, 179–223.

Davis, L. D. (1973). *Circulation Res.* **32**, 206–214.

Deck, K. A., Kern, R. & Trautwein, W. (1964). *Pflügers Arch. ges. Physiol.* **280**, 50–62.

Ferrier, G. R. & Moe, G. K. (1973). *Circulation Res.* **33**, 508–515.

Tsien, R. W. (1974). *J. gen. Physiol.* **64**, 293–319.

The influence of extracellular potassium ions on the action of ouabain on membrane currents in sheep Purkinje fibres

By I. Cohen, J. Daut and D. Noble. *University Laboratory of Physiology, South Parks Road, Oxford*

It is well known that the toxic effects of cardiac glycosides may be countered by increasing plasma K^+ (see e.g. Scherf & Schott, 1973). We have studied the influence of K^+ ions on the action of ouabain in voltage-clamped Purkinje fibres. Our results show that current changes attributable to pump blockage, which may induce abnormal rhythms, are indeed countered by increasing $[K]_o$. However, at $[K]_o$ levels sufficient to prevent ouabain-induced potassium loss ouabain was found to have a quite different action on the membrane current.

This is illustrated in Fig. 1. At 5·4 mM $[K]_o$ (or higher) 5×10^{-7} M ouabain reduces the net inward current required to hyperpolarize the membrane. When the same fibre was exposed to 4 mM $[K]_o$, ouabain shifted the current level in an inward direction. The latter effect would be expected if ouabain were to act by blocking an outward electrogenic current (cf. Isenberg & Trautwein, 1974), but a similar change would also be expected if the cell were to lose potassium. A reversible positive change in E_K does occur in our experiments and accounts for some, at least, of the current change near E_K. By contrast, the effects observed at the higher $[K]_o$ levels are unexpected. They are usually accompanied by a small hyperpolarization and a negative shift in E_K. This may be produced by net stimulation of the pump, and since it occurs at doses in the therapeutic range it may be of clinical significance.

The ouabain doses required to produce these effects appear to depend on the potassium concentration in the bathing solution. For example the effects attributable to pump blockage occurred at ouabain concentrations $> 1 \times 10^{-7}$ M in 2·7 mM-K^+ Tyrode, but a ouabain concentration of 5×10^{-7} M was required to produce the same effect in 4 mM-K^+. Our results are consistent with the view that there is competition between K^+ and the cardiac glycoside for an external membrane site (cf. Glynn, 1957).

We should like to acknowledge the support of the Muscular Dystrophy Association of America, the Rhodes Foundation and the Medical Research Council.

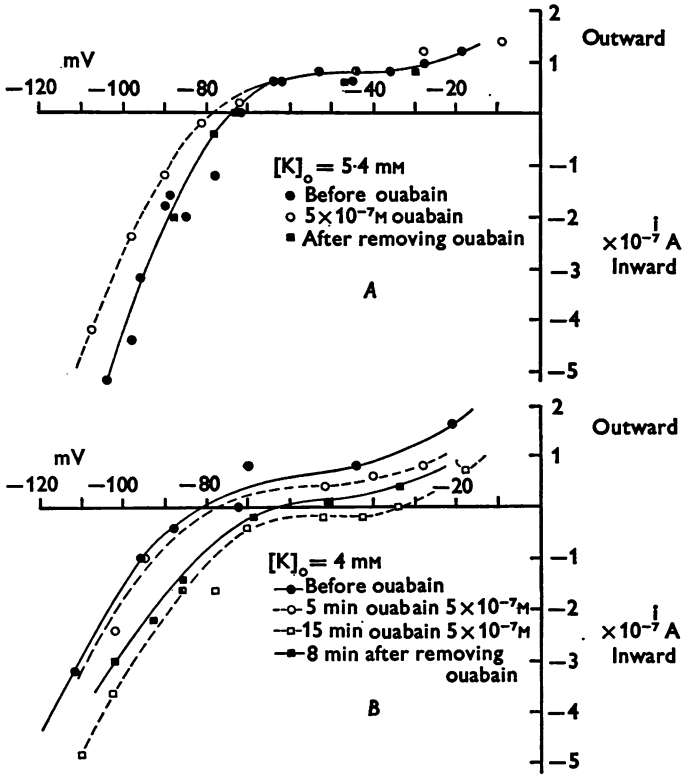

Fig. 1. *A*, steady-state current–voltage relations of sheep Purkinje fibre exposed to 5·4 mM-K^+. Addition of 5×10^{-7} M ouabain produces a small (5 mV) hyperpolarization and a substantial decrease in the current required to hyperpolarize the membrane. Note complete recovery on removing ouabain. The ouabain dose was chosen to be between the therapeutic and toxic ranges (cf. Tuttle, Witt & Farah, 1961). *B*, steady-state current–voltage relations of same fibre exposed to 4 mM-K^+. Ouabain now produces a large depolarization and the current required to hyperpolarize the membrane is increased. Only partial recovery was observed. More complete recovery was observed in some fibres. Note also that in this fibre the current shifts in an inward direction at all potentials (cf. Isenberg & Trautwein, 1974). In most fibres we found that ouabain and K^+ concentrations that produce an inward shift at hyperpolarized potentials produced an outward shift at depolarized potentials (i.e. the ouabain curve crosses the normal curve).

REFERENCES

Glynn, I. M. (1957). *J. Physiol.* **136**, 148–173.

Isenberg, G. & Trautwein, W. (1974). *Pflügers Arch. ges. Physiol.* **350**, 41–54.

Scherf, D. & Schott, A. (1973). *Extrasystoles and Allied Arrhythmias*, ch. 12. London: Heineman.

Tuttle, R. S., Witt, P. N. & Farah, A. (1961). *J. Pharmac. exp. Ther.* **133**, 281.

The effect of glucose on fluid readsorption in rat renal proximal convoluted tubules

By J. H. V. Bishop, R. Green and S. Thomas. *Department of Physiology, University of Manchester, Manchester M13 9PT*

Recently, evidence has been presented that the presence of glucose in proximal tubular fluid might be expected to influence sodium transport in a number of ways, and secondarily affect net fluid transport (see Frömter, Rumrich & Ullrich, 1973; Frömter & Gessner, 1974).

To investigate this possibility a direct micropuncture study of sodium, potassium, glucose and fluid transport was undertaken by continuously perfusing, at 32 nl. min^{-1}, randomly selected proximal convoluted tubules with solutions containing 0 or 5 mM.$l.^{-1}$ glucose. The ionic composition of the non-glucose containing solution was (in mM.$l.^{-1}$) NaCl, 154; KCl, 4; $NaHPO_4$, 2; KH_2PO_4, 0·5; and $CaCl_2$, 2. When 5 mM.$l.^{-1}$ glucose was added, the sodium chloride concentration was reduced to 151·3 mM.$l.^{-1}$. The measured osmolality of both solutions was 303 m-osmole.kg $water^{-1}$ and the pH 7·40. Sodium, potassium, glucose and osmolal concentrations in collected and perfusion fluids were measured, as was the [^{3}H]methoxy inulin concentration, and net fluxes were calculated.

With the glucose-free perfusion solution glucose appeared in the collected perfusate at a concentration of 0·90 ± 0·06 mM.$l.^{-1}$ (mean ± S.E. of mean), representing a net flux into the tubule of 23 ± 2 p-mole.min^{-1}.mm $tubule^{-1}$ (mean ± S.E. of mean). On the basis of published values for the permeability of the epithelium to glucose (e.g. von Baeyer, von Conta, Haeberle & Deetjen, 1973) this flux exceeds that which can be accounted for by passive diffusion; and it suggests that the carrier-mediated mechanism for glucose is working in reverse. With glucose present in the tubular perfusate a net re-absorption of 27 ± 2 p-mole.min^{-1}.mm $tubule^{-1}$ resulted.

The net fluid fluxes from the lumen in the two series did not differ significantly, the flux being 3·23 ± 0·24 ml.min^{-1}.mm $tubule^{-1}$ when glucose was absent and 2·97 ± 0·12 nl. min^{-1}.mm $tubule^{-1}$ when glucose was present; nor was there a significant difference between the net sodium fluxes.

We conclude that, under free-flow conditions, the presence or absence of glucose makes no significant difference to the net re-absorption of sodium or fluid in the proximal convoluted tubule.

This work was supported in part by M.R.C. grant G 973/253/B.

REFERENCES

FRÖMTER, E. & GESSNER, K. (1974). *Pflügers Arch. ges. Physiol.* **351**, 85–98.

FRÖMTER, E., RUMRICH, G. & ULLRICH, K. J. (1973). *Pflügers Arch. ges. Physiol.* **343**, 189–220.

VON BAEYER, H., VON CONTA, C., HAEBERLE, D. & DEETJEN, P. (1973). *Pflügers Arch. ges. Physiol.* **343**, 273–286.

A note on the split-droplet technique as applied to proximal tubules of the rat kidney

BY C. LE GRIMELLEC, P. POUJEOL and D. G. SHIRLEY.* *Département de Biologie, C.E.N. de Saclay, 91 Gif-sur-Yvette, France*

During an investigation into ionic fluxes in the proximal tubule of the Munich strain Wistar rat, a modification of the Gertz split-oil-droplet technique, in which a column of castor oil deposited in a tubule is split by a fluid droplet (Gertz, 1963), was used. Each injected fluid droplet was re-aspirated following a variable period of time (up to 42 sec), and its ionic content determined using electron probe analysis. [^{3}H]methoxy inulin was used as a marker of fluid movement, the ratio of the inulin activities in collected and injected samples (c/i In) being measured for each droplet. When fluid of composition 125 m-equiv/l. NaCl, 4 m-equiv/l. KCl (isotonicity maintained by addition of sucrose) was used as the injectate, there was negligible transepithelial net movement of fluid; the regression equation relating the c/i In ratio (Y) to time in sec (X) was

$$Y = 0{\cdot}99 - 0{\cdot}003X \quad (n = 25).$$

Whenever it was possible to re-aspirate all of the injected solution, the volume of the collected sample was determined. The mean value of this volume was only 75·5% ($\pm$ 2·7 S.E. of mean, $n = 14$) of the volume injected.

Another unexpected observation was that when these experiments were performed during intravenous infusion of sodium ferrocyanide, all the reaspirated split-droplet samples contained some ferrocyanide: comparison of the Fe concentration in the samples with that of the glomerular filtrate of corresponding tubules showed the mean sample/glomerulus Fe ratio to be 0·59 $\pm$ 0·09 (S.E. of mean, $n = 7$).

From the results with inulin it seemed unlikely that the presence of ferrocyanide resulted from samples being contaminated by tubular fluid. Nevertheless, this possibility was examined in a separate series of experiments by injecting a 100 μl. pulse containing 100 μCi [^{3}H]methoxy inulin

* Present address: Department of Physiology, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF.

into the aorta just above the renal arteries during the split-droplet procedure *after* the oil column had been split by the injectate. (In these experiments inulin was not added to the injected fluid droplet.) It was found that the re-aspirated samples contained no inulin (signal : background ratio = $1{\cdot}00 \pm 0{\cdot}03$ S.E. of mean, $n = 9$) whereas tubular fluid collected immediately proximal to the oil columns contained significant amounts of inulin (signal : background ratio = $2{\cdot}63 \pm 0{\cdot}30$ S.E. of mean, $n = 9$).

The results of these experiments show that when an isotonic solution containing 125 m-equiv NaCl/l. is placed in the proximal tubule, there is no net fluid movement across the tubular wall. Further, in order to account for the presence of the ferrocyanide in reaspirated samples and the apparent reduction in the volume of the injectate, it is suggested that in the split-droplet experimental procedure the oil column may not percolate the spaces between microvilli of proximal tubular cells, resulting in the trapping of some tubular fluid.

REFERENCE

GERTZ, K. H. (1963). *Pflügers Arch. ges Physiol.* **276**, 336.

Cardiovascular responses and renin release during stimulation of afferent renal nerves in the cat

BY F. CAFIERO, F. R. CALARESU,* A. STELLA and A. ZANCHETTI. *Istituto Ricerche Cardiovascolari, Universita di Milano, via Sforza 35 and CNR, Milano, Italy*

As stimulation of afferent renal nerves in the rabbit (Aars & Akre, 1970) and occlusion of the renal vein in the cat (Beacham & Kunze, 1969) have been reported to elicit arterial hypotension, the existence of renal arterial baroreceptors with activity similar to that of sino-aortic baroreceptors (Niijima, 1971) has been suggested. Recently it has also been proposed that renal afferent fibres may be involved in a reflex arc leading to release of renin (Zanchetti & Stella, 1975). The present study was done to investigate the effect of stimulation of afferent renal nerves on haemodynamic variables and on the release of renin in the cat.

Identical results were obtained from 12 cats anaesthetized with sodium pentobarbital (40 mg/kg I.P.) and three cats with alpha-chloralose (60 mg/kg I.V.). Renal nerves on either side were crushed distally and stimulated through sleeve electrodes. Ipsilateral and contralateral renal blood flows, mesenteric and iliac blood flows were measured electro-magnetically and recorded together with arterial pressure and heart rate. Renin release

* M.R.C. of Canada Visiting Professor.

from both kidneys was determined as described previously (Richardson, Stella, Leonetti, Bartorelli & Zanchetti, 1974).

Stimulation of afferent renal nerves for 20–30 sec elicited a moderate increase in arterial pressure and a very slight increase in heart rate, no change in contralateral renal blood flow, and a decrease in mesenteric and iliac blood flows (Table 1). This pattern of responses was present at all parameters of stimulation used (0·05–5 msec, 5–50 Hz, 5–30 V), and after

TABLE 1. Means and percentage changes (± S.E. of mean) of haemodynamic responses to electrical stimulation of afferent renal nerves compared to control values. *P* values refer to differences between means, *n* indicates number of experimental runs

	n	Control	Response	*P*	% change from control
Arterial pressure (mmHg)	25	119·4 ± 3·6	138·4 ± 3·5	< 0·001	17 ± 2
Heart rate (beats/min)	15	208·5 ± 9·4	214·3 ± 9·4	< 0·001	3 ± 1
Contralateral renal flow (ml./min)	16	23·5 ± 1·7	24·1 ± 1·8	> 0·05	2 ± 1
Sup. mesenteric flow (ml./min)	14	22·5 ± 0·8	15·4 ± 1·3	< 0·001	−32 ± 4
Iliac flow (ml./min)	14	15·0 ± 1·2	12·0 ± 1·0	< 0·001	−20 ± 1

administration of gallamine. Renin release was found not to be significantly altered by 5 min stimulation of afferent renal nerves (from 22 ± 15 to 36 ± 16 ng/min in the denervated kidney and from 58 ± 12 to 61 ± 13 ng/min in the contralateral innervated kidney in eight experiments).

We conclude that stimulation of afferent renal nerves elicits an increase in arterial pressure, which appears to be due to widespread vasoconstriction, but does not alter renin release. This reflex response cannot be accounted for by activation of baroreceptors; the receptors involved remain to be determined, especially since our results in the cat are at variance with those reported in the rabbit (Aars & Akre, 1970).

REFERENCES

AARS, H. & AKRE, S. (1970). *Acta physiol. scand.* **78**, 184–188.
BEACHAM, W. S. & KUNZE, DIANA L. (1969). *J. Physiol.* **201**, 73–85.
NIIJIMA, A. (1971). *J. Physiol.* **219**, 477–485.
RICHARDSON, D., STELLA, A., LEONETTI, G., BARTORELLI, A. & ZANCHETTI, A. (1974). *Circulation Res.* **34**, 425–434.
ZANCHETTI, A. & STELLA, A. (1975). *Clin. Sci.* (in the Press).

Release of amino acids from the muscles of normal and fasted rabbits following injection of glucagon

By P. M. Daniel, O. E. Pratt and E. Spargo. *Department of Neuropathology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF*

Fasting raises the level of glucagon in the blood. This hormone has a regulatory role in amino acid metabolism (Marliss, Aoki & Cahill, 1972). We present evidence for a function of glucagon in fasting.

Under pentobarbitone anaesthesia catheters for sampling blood were inserted into an artery, into the vein draining the thigh muscles, and one for injecting glucagon into the femoral artery above these muscles. Arterial and venous blood samples were taken simultaneously at 15 min intervals before and during a continuous intra-arterial injection of glucagon by an electronically controlled syringe (Pratt, 1974) at a rate programmed (Daniel, Donaldson & Pratt, 1975) to maintain a high concentration of glucagon in the plasma (1–2 μg ml.$^{-1}$).

As the arterial blood glucose (whole blood) rose during the glucagon injection (Fig. 1), the total α-amino nitrogen in the arterial blood plasma

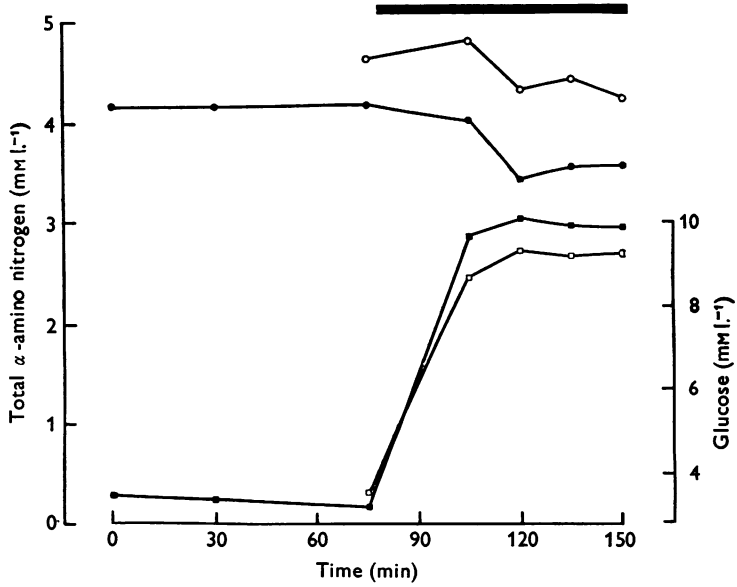

Fig. 1. Shows the change in the concentration of total plasma α-amino nitrogen, as well as that in the concentration of glucose, in the arterial blood and in the blood draining from the muscles during an injection of glucagon into a rabbit fasted for 3 days. Total plasma α-amino nitrogen in arterial blood, ●; total plasma α-amino nitrogen in venous blood from muscles, ○; blood glucose in arterial blood, ■; blood glucose in venous blood from muscles, □; filled bar, injection of glucagon.

fell, the fall varying from 0·1 to 0·75 mM ($P < 0·001$, t-test). The fall was greater in four fasted (3 days) than in four fed animals, $0·58 \pm 0·07$ and $0·29 \pm 0·11$ mM respectively, mean ± S.E. of mean ($P < 0·05$). Concomitantly release of α-amino nitrogen from the muscles was doubled in the fasted animals ($P < 0·001$). This additional nitrogen output was accounted for mainly by an increased release of L-glutamine, glycine, L-alanine and L-methionine from the muscles. In spite of this increased release the concentrations of these amino acids fell in the arterial plasma. Simultaneously the muscles began to take up glucose, as shown by a change in the arteriovenous difference from $-0·32 \pm 0·02$ to $+0·81 \pm 0·28$ mM ($P < 0·05$).

In fed animals the glucose that is mobilized when glucagon is given probably comes mainly from glycogen stores but in fasted animals it appears to come largely from blood amino acids which are broken down by the liver to make glucose. These blood amino acids appear to be continuously replenished by a sustained release of free amino acids from the muscles, which comprise 45% of the body mass.

REFERENCES

DANIEL, P. M., DONALDSON, J. & PRATT, O. E. (1975). *Med. & Biol. Engng* **13**, 214–227.

MARLISS, T., AOKI, T. T. & CAHILL, G. F. JR. (1972). In *Glucagon*, ed. LEFEBVRE, P. J. & UNGER, R. H., ch. 8. Oxford: Pergamon Press.

PRATT, O. E. (1974). *J. Physiol.* **237**, 5–6*P*.

Accumulation of D-galactose at the serosal border of rabbit ileum: the effects of theophylline

BY G. D. HOLMAN and R. J. NAFTALIN. *Department of Physiology, University of Leicester, Leicester LE1 7RH*

Increased L-leucine accumulation into rat jejunum has been observed in response to theophylline and di-butyryl cyclic AMP. (Kinzie, Ferrendelli & Alpers, 1973). Recently Burrill, Sattelmeyer & Lerner (1974) showed that theophylline increased L-methionine influx into Na-depleted jejunum. Theophylline is known to reverse net Na and chloride flux across mammalian ileum (Powell, Binder & Curran, 1972; Nellans, Frizzell & Schultz, 1973; Al-Awqati, Cameron & Greenough, 1973). Hence, it is of interest to observe how the drug affects Na-dependent galactose transport across rabbit ileum.

The effect of theophylline on the unidirectional fluxes of galactose across the mucosal and serosal borders of rabbit ileum and on steady-state tissue accumulation was determined using the method described by Naftalin & Curran (1974) and Naftalin & Holman (1974). This involves simultaneous measurement of the steady-state bidirectional fluxes of ^{3}H-labelled

TABLE 1. The experiments were carried out in Ringer bicarbonate gassed with 95% O_2–5% CO_2 at 37° C containing 2 mM galactose. The incubation period was 90 min. The significance levels for the theophylline effects were assessed using one-way analysis of variance and for di-butyryl cyclic AMP using Student's t test (unpaired means solution). All figures are expressed as means ± S.E. of mean. The unidirectional fluxes, J_{ij} (where components 1, 2, 3 are respectively mucosal solution, tissue water and serosal solution) are calculated from the following relationships:

$$J_{12} = J_{31}R + J_{13};\ J_{21} = J_{31}(1+R);\ J_{23} = J_{13}(1+1/R);\ J_{32} = J_{31} + J_{13}/R.$$

The unidirectional permeabilities $P_{ij} = J_{ij}/C_i$.

		μmole cm^2 hr^{-1}				Accumulation	cm hr^{-1}				
	n	J_{13}	J_{31}	J_{net}	R	(mM)	P_{12}	P_{21}	P_{23}	P_{32}	$\ln \frac{P_{12}}{P_{21}}$
Control	4	0·294 ± 0·034	0·013 ± 0·0047	0·259 ± 0·045	3·32 ± 0·47	11·51 ± 1·4	0·131 ± 0·02	0·0048 ± 0·001	0·0362 ± 0·0042	0·055 ± 0·0047	3·51 ± 0·306
Theophylline											
0·1 mM	4	0·282 ± 0·016	0·0125 ± 0·0028	0·227 ± 0·041	4·32 ± 0·36	12·77 ± 0·825	0·124 ± 0·026	0·0047 ± 0·0008	0·0275 ± 0·0022	0·04 ± 0·0034	3·38 ± 0·266
1·0 mM	4	0·255 ± 0·058	0·0107 ± 0·0031	0·211 ± 0·073	6·63 ± 0·88	16·54 ± 1·02	0·136 ± 0·041	0·0043 ± 0·0008	0·0207 ± 0·0039	0·03 ± 0·0065	3·34 ± 0·34
5·0 mM	4	0·177 ± 0·031	0·0095 ± 0·0038	0·149 ± 0·044	10·78 ± 1·34	20·46 ± 1·26	0·158 ± 0·058	0·0055 ± 0·0016	0·0105 ± 0·0017	0·015 ± 0·0028	3·07 ± 0·414
10·0 mM	4	0·144 0·015	0·0072 ± 0·0034	0·142 ± 0·0122	11·16 ± 1·29	19·91 ± 1·83	0·154 ± 0·019	0·0054 ± 0·0019	0·0105 ± 0·0021	0·013 ± 0·0034	3·24 ± 0·327
P		< 0·01	< 0·05	< 0·05	< 0·001	< 0·001	n.s.	n.s.	< 0·001	< 0·001	n.s.
Di-butyryl cyclic AMP											
1·0 mM	3	0·18 ± 0·004	0·0023 ± 0·0008	0·177 ± 0·0038	10·32 ± 0·54	26·48 ± 1·39	0·105 ± 0·005	0·0011 ± 0·0004	0·007 ± 0·00001	0·0096 ± 0·0003	4·57 ± 0·33
P		< 0·05	< 0·01	n.s.	< 0·001	< 0·001	n.s.	< 0·05	< 0·001	< 0·001	n.s.

D-galactose from mucosal–serosal sides and ^{14}C-labelled D-galactose in the opposite direction. The ratio R of $^{3}H/^{14}C$-labelled galactose and the total amount of galactose in the tissue water following incubation are also measured.

The data shown in Table 1 reveal that theophylline reduces both the bidirectional transmural flux and net galactose absorption. Theophylline also causes a threefold increase in both the ratio R and galactose accumulation. The derived permeabilities indicate that theophylline reduces galactose flux across the serosal border without affecting brush-border galactose transport.

Since galactose accumulation is increased without concurrent change in mucosal transport or in the permeability ratio of the serosal border to galactose, these findings are consistent with the view, previously stated, (Holman & Naftalin, 1975) that, although the permeability asymmetry required for net galactose absorption is generated at the brush-border, *accumulation* within the tissue results from retardation of galactose exit at the serosal border, with consequent concentration polarization within the epithelial cells.

The known action of theophylline and di-butyryl cyclic AMP in inhibiting phosphodiesterase (EC 3.1.4.17) activity suggests that the permeability of the serosal border to galactose may be affected by the tissue levels of 3′,5′-cyclic AMP.

The authors wish to thank the M.R.C. for financial support.

REFERENCES

Al-Awqati, Q., Cameron, J. L. & Greenough, W. B. (1973). *Am. J. Physiol.* **224**, 818–823.

Burrill, P. H., Sattelmeyer, P. A. & Lerner, J. (1974). *Biochim. biophys. Acta* **373**, 265–276.

Holman, G. D. & Naftalin, R. J. (1975). *Biochim. biophys. Acta* **382**, 230–245.

Kinzie, J. L., Ferrendelli, J. A. & Alpers, D. H. (1973). *J. biol. Chem.* **248**, 7018–7024.

Naftalin, R. J. & Curran, P. F. (1974). *J. membrane Biol.* **16**, 257–278.

Naftalin, R. J. & Holman, G. D. (1974). *Biochim. biophys. Acta* **373**, 453–470.

Nellans, H. N., Frizzell, R. A. & Schultz, S. G. (1973). *Am. J. Physiol.* **225**, 467–475.

Powell, D. W., Binder, H. J. & Curran, P. F. (1972). *Am. J. Physiol.* **224**, 191–196.

The sensitivity of chronically de-efferented muscle spindles to acetylcholine

By G. L. Kidd and J. Kučera.* *Department of Physiology, The University, Liverpool L69 3BX and Department of Neurology, Washington University, St Louis, U.S.A.*

Chemical supersensitivity following chronic denervation occurs in many tissues, and muscle spindles are no exception (Arutyunyan, 1969). We have examined the effects of acetylcholine (ACh) on acutely and chronically de-efferented spindles employing the rat tail-base muscle preparation (Gladden & Kidd, 1969) to establish the minimal effective concentration of the agent acting under the two conditions.

Acetylcholine is a spindle excitant (Hunt, 1952) which is presumed to act largely via the intrafusal muscle fibres (Smith & Albuquerque, 1967). It was added to a Krebs solution bathing the exposed muscle, and the evoked afferent activity from primary and secondary spindle endings was recorded from dorsal roots. In the acutely de-efferented preparation, the minimal effective concentration of ACh to induce maintained activity in spindles stretched to just below their mechanical threshold was $2{\cdot}5$–$5{\cdot}0 \times 10^{-8}$ g/ml., with the primary and secondary endings responding similarly.

With the chronic preparation, in which the ventral roots had been sectioned under Nembutal anaesthesia (1·5 ml./100 g) and allowed to degenerate for 20–22 days, the minimal effective concentration was reduced to $1{\cdot}0$–$2{\cdot}0 \times 10^{-8}$ g/ml. Once again, the primary and secondary endings showed similar responsiveness. In both forms of preparation, the evoked spindle activity persisted for as long as the ACh remained in the pool (usually 10 min) and it was antagonized by tubocurarine ($1{\cdot}0 \times 10^{-6}$ g/ml.).

In all the experiments, ACh was tested in the presence of neostigmine bromide ($2{\cdot}0 \times 10^{-6}$ g/ml.); this substance provoked irregular, transient bursts of activity from acutely de-efferented spindles, but it had no effect on those subjected to chronic de-efferentation.

The chronically de-efferented extrafusal muscle fibres did not begin to develop the anticipated transient contracture until the concentration of ACh was increased to $1{\cdot}0 \times 10^{-7}$ g/ml. We were able, therefore, to study the response of spindles to ACh uninfluenced by mechanical disturbances arising in the skeletal muscle.

Assuming that the ACh acted principally via the intrafusal muscle fibres, we conclude that after 20–22 days of chronic de-efferentation they showed a 2·5–5·0 times increase in sensitivity to the agent. However, the

* George Holt Fellow, gratefully acknowledging financial assistance from the Wellcome Trust.

intrafusal fibres became 10 times more sensitive to ACh than de-efferented extrafusal muscle.

The slight sensitivity change of intrafusal relative to extrafusal muscle compares with their retention of structural integrity following chronic de-efferentation (cf. Barker, 1974). The similar sensitivities to ACh of primary and secondary spindle endings prior to and following chronic de-efferentation is presumably indicative of the sensitivity of nuclear bag and nuclear chain fibres and is interesting in view of the evidence that both forms of muscle fibre share part of their efferent innervation (cf. Barker, 1974).

REFERENCES

ARUTYUNYAN, R. S. (1969). *Bull. exp. Biol. Med. U.S.S.R.* **68**, 14–17.
BARKER, D. (1974). In *Handbook of Sensory Physiology*, vol. III/2, *Muscle Receptors*, ed. HUNT, C. C., pp. 1–95. New York: Springer-Verlag.
GLADDEN, M. H. & KIDD, G. L. (1969). *J. appl. Physiol.* **26**, 507–509.
HUNT, C. C. (1952). *Fedn Proc.* **11**, 75.
SMITH, C. M. & ALBUQUERQUE, E. X. (1967). *J. Pharmac. exp. Ther.* **156**, 573–584.

The dynamic responses of Golgi tendon organs

BY E. K. STAUFFER and J. A. STEPHENS.* *Department of Physiology, College of Medicine, University of Arizona, Tucson, Arizona 85724, U.S.A.*

The dynamic responses of 37 soleus tendon organs have been studied during ramp changes of force development produced by whole muscle and motor unit contractions, in 13 cats anaesthetized with pentobarbitone sodium.

The discharges of single Ib afferent fibres were recorded from subdivided dorsal rootlets and identified on the basis of their accelerated discharge during a twitch or short tetanus of the whole muscle. Single motor units whose contraction elicited tendon organ firing were functionally isolated by subdividing ventral rootlets. Contractions of the whole soleus muscle were produced by stimulating ventral roots L7 and S1. Motor units and whole muscles were stimulated at 100 pulses/sec.

Ramp changes in force development were produced by allowing the muscle to contract against a servo-regulated electromagnetic puller arranged in force servo (for details see Reinking & Stuart, 1974). The accompanying changes in muscle length were limited to 15 mm and took place over the ascending limb of the muscle length tension curve.

The dynamic response of a tendon organ was assessed using a dynamic index (D.I.) defined as the peak discharge frequency occurring at the end of the rising phase of a ramp, minus the frequency 0·5 sec later.

* Present address: The Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH.

For whole muscle and motor unit contractions the ranges of velocities used were 1–100 kg/sec and 1–500 g/sec respectively.

During both whole muscle and motor unit contractions the relationship between D.I. and rate of change of force ($\dot{F}$) for each receptor could be described by a power function of the form D.I. $= a.\dot{F}^b$, where $0 < b < 1$. In these cases where the tendon organ response could be measured during negative rates of change of force, the regression lines for positive and negative velocities were symmetrical. The average dynamic responses recorded during whole muscle and motor unit contractions were 67 ± 39 and $10 \pm$ S.E. of mean and 3 p.p.s. respectively.

No initial burst or acceleration-like discharges were observed at the onset of the ramps.

The size of the dynamic response increased with more prolonged ramp changes in force but decreased as the starting value of force increased.

When the behaviour of a single tendon organ was compared during the same rate of force development by various motor units, different dynamic responses were observed. This finding emphasizes that the response of a tendon organ during single motor unit contractions is determined by the force exerted in series with the receptor capsule rather than the total force exerted at the tendon (cf. Stephens & Stuart, 1974).

In many respects it is convenient to summarize the dynamic behaviour of tendon organs as being similar to the responses to muscle spindle secondary endings during ramp changes in length.

This work was supported in part by U.S.P.H.S. grants NS 07888 and HE 5884.

REFERENCES

Reinking, R. M. & Stuart, D. G. (1974). *Am. J. phys. Med.* **53**, 1–28.
Stephens, J. A. & Stuart, D. G. (1974). *J. Physiol.* **242**, 62–63*P*.

Facilitation of the tonic vibration reflex in the spinal cat by 5-hydroxytryptophan (5-HTP)

By P. H. Ellaway and Judy R. Trott. *Department of Physiology, University College London, Gower Street, London WC1E 6BT*

Vibration applied to the tendon of the soleus muscle at amplitudes of 50–100 μm and frequencies of 50–200 Hz evokes, in the decerebrated cat, a reflex tonic contraction in the muscle. This contraction cannot be elicited once the spinal cord has been sectioned (Matthews, 1966). The primary endings of muscle spindles have been shown to be the receptors responsible for the tonic vibration reflex seen in the decerebrated preparation (Brown, Engberg & Matthews, 1967).

We have treated the unanaesthetized cat, whose spinal cord has been

transected in the mid-thoracic region, with 5-HTP (50 mg/kg I.V.). A tonic vibration reflex typical of the decerebrated preparation could then be elicited in triceps surae muscles some 10–20 min after injection. The muscle selected (usually soleus) was initially stretched to a length a few millimetres short of natural full extension to ensure that vibration excited primary endings maximally. Apart from the muscle concerned the limb was denervated below the hip. Typical experimental results are shown in Fig. 1.

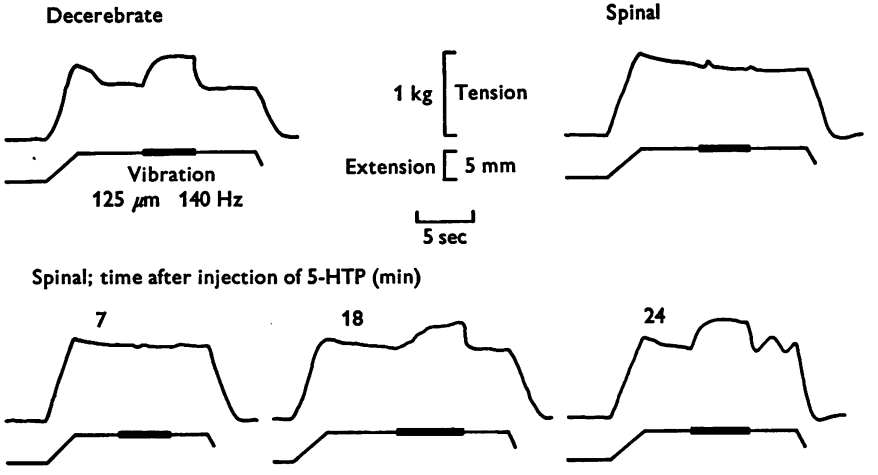

Fig. 1. Reflex responses of the soleus muscle to vibration applied to the Achilles tendon after stretching the muscle 5 mm from a resting position (approx. 10 mm below maximum physiological extension). In all records stretch causes a considerable increase in passive tension but only in the decerebrate is there any active tension due to a stretch reflex (it lasts 2·5 sec after peak tension is reached). Top left: the tonic vibration reflex in the decerebrate cat. Top right: absence of the reflex following spinal section. Bottom traces: gradual reappearance of the reflex response to vibration following injection of 5-HTP (50 mg/kg).

The drug 5-HTP, which crosses the blood–brain barrier, is thought to cause release of neurotransmitter (Lundberg, 1965) from terminals, known to occur in the lumbar spinal cord, of descending 5-hydroxytryptaminergic nerve tracts (Carlsson, Falck, Fuxe & Hillarp, 1964). We suggest that the increased release of neurotransmitter potentiates the tonic vibration reflex by facilitating transmission from group Ia afferents of primary spindle endings to alpha motoneurones rather than by exciting gamma motoneurones. The reflex response would be independent of gamma activity under the present experimental conditions where the muscle is extended since, at long muscle lengths, vibration excites primary endings at the frequency of vibration irrespective of background gamma activity (Brown

et al. 1967). This work supports our previous findings where 5-HTP was found to facilitate the stretch reflex independently of its excitatory action on gamma motoneurones (Ellaway & Trott, 1974, 1975).

We are grateful to the M.R.C. for financial support of one of us (J.R.T.) and for the provision of recording equipment.

REFERENCES

BROWN, M. C., ENGBERG, I. & MATTHEWS, P. B. C. (1967). *J. Physiol.* **192**, 773–800.
CARLSSON, A., FALCK, B., FUXE, K. & HILLARP, N.-Å. (1964). *Acta physiol. scand.* **60**, 112–119.
ELLAWAY, P. H. & TROTT, J. R. (1974). Participation of gamma motoneurones in the stretch reflex produced by 5-HTP in the spinal cat. *J. Physiol.* **239**, 56–57*P*.
ELLAWAY, P. H. & TROTT, J. R. (1975). *Expl Brain Res.* **22**, 145–162.
LUNDBERG, A. (1965). In *Studies in Physiology*, ed. CURTIS, D. R. & MCINTYRE, A. K., pp. 186–190. Berlin, Heidelberg, New York: Springer.
MATTHEWS, P. B. C. (1966). *J. Physiol.* **184**, 450–472.

On the response linearity of neurones in cat visual cortex

BY J. A. MOVSHON and D. J. TOLHURST. *Psychological and Physiological Laboratories, University of Cambridge, Cambridge CB2 3EB*

Hubel & Wiesel's (1962) description of the receptive fields of cat visual cortical neurones suggests that simple cells may behave linearly, whereas complex cells are clearly non-linear. But Maffei & Fiorentini (1973) have reported that the modulation depth of the response of simple cells to drifting gratings is proportional to the *logarithm* of the grating's contrast. If simple cells were linear, the response would be directly proportional to the contrast.

We recorded extracellularly from neurones in the striate cortex of adult cats, using conventional techniques (see Movshon, 1975). Receptive fields were classified as simple or complex by the criteria of Hubel & Wiesel (1962). Drifting gratings of sinusoidal luminance profile, optimized for orientation and direction of movement, were generated by a PDP 11/20 computer and displayed on the face of an oscilloscope of mean luminance 150 cd m^{-2}, subtending 12·5° by 10° at a distance of 114 cm from the cat's eyes. Artificial pupils (3 mm diam.) were used, and stimuli presented monocularly. The computer also compiled histograms of the responses to successive cycles of the grating. Stimuli of nine different contrasts and a blank were presented for 10 sec each in random order to form one block of trials; the response to five blocks was accumulated for each cell, representing at each contrast the response to 100–200 cycles of the grating.

The responses of simple cells to drifting gratings were modulated in synchrony with the stimulus. The response wave form was not sinusoidal but appeared to be half-wave rectified since the resting discharge in these

cells was low or absent. The responses of complex cells were usually unmodulated, but some cells gave a modulated discharge to gratings, superimposed on an unmodulated elevation of firing rate; the modulation, when observed, was most prominent at low spatial frequencies. In this respect, the behaviour of simple cells is reminiscent of that of the cat's X-type retinal ganglion cells; complex cells resemble Y-cells (Enroth-Cugell & Robson, 1966).

Eleven cells (eight simple and three complex) gave modulated responses at some spatial frequency; in each case, the modulation depth was linearly related to contrast but saturated at Michelson contrasts above about 0·3. Six complex cells gave unmodulated responses. In five cases the increase in discharge was approximately proportional to the square or cube of contrast and saturated at relatively low contrasts. In one case, the response seemed linearly related to contrast.

The amplitudes of the *modulated* responses of both cell types appear to be linearly related to contrast. Complex cells also show an *unmodulated* elevation of discharge (a non-linearity in itself), whose amplitude is usually not linearly related to contrast. Simple cells appear to be linear, apart from a half-wave rectification.

J.A.M. holds a Research Training Scholarship from the Wellcome Trust. The work was supported by a grant (no. G/972/463B) to C. Blakemore from the Medical Research Council, which also provided the computer.

REFERENCES

ENROTH-CUGELL, C. & ROBSON, J. G. (1966). *J. Physiol.* **187**, 517–552.
HUBEL, D. H. & WIESEL, T. N. (1962). *J. Physiol.* **160**, 106–154.
MAFFEI, L. & FIORENTINI, A. (1973). *Vision Res.* **13**, 1255–1267.
MOVSHON, J. A. (1975). *J. Physiol.* **249** (3) (in the Press).

Properties of individual components of pattern-onset evoked potentials in man

BY C. R. JAMES and D. A. JEFFREYS. *Department of Communication, The University of Keele, Newcastle, Staffs., ST5 5BG*

The averaged VEPs (visual evoked potentials) recorded from the human scalp to the onset of a stationary stimulus pattern at low temporal frequencies (< 2 Hz) contain three distinct pattern-specific components. For high-contrast patterns these components, C. I, C. II and C. III, have peak latencies of about 75, 100 and 150 msec, respectively, and for relatively short stimulus durations (e.g. 25 msec), they generally have the largest recordable amplitudes (up to 30 μV) of any stimulus-related VEP components. They are independent of the VEPs produced by changes of over-all luminance.

Jeffreys & Axford (1972) have described how the likely sites of origin of these components can be predicted from their surface amplitude distributions and their systematic variation with the retinal location of the pattern. The distribution characteristics observed have shown that the three components have anatomically distinct cortical origins and indicate that C. I originates in striate cortex and C. II and C. III in different regions of extrastriate cortex (areas 18 and/or 19).

Subsequent experiments have demonstrated marked differences in the stimulus-related characteristics of C. II and C. III compared with C. I, thus indicating different properties for the underlying physiological processes in extrastriate and striate cortex. The main findings of these experiments are:

(1) Corresponding patterns of positive or negative contrast produce similar VEPs.

(2) Grid patterns are relatively ineffective stimuli for C. II and C. III which are mainly sensitive to discontinuous contours.

(3) C. II and C. III are more sensitive to the sharpness of the outlines of the stimulus pattern than C. I.

(4) C. II and C. III, but not C. I, can be greatly attenuated by the presence of steady outlines which either match the edges of the pattern elements or are adjacent and of similar orientation and dimensions.

(5) C. II and C. III, unlike C. I, are attenuated by quite brief pre-exposures of the stimulus pattern. This adaptation, which is at least partly orientation-specific, occurs also with successive presentations of the stimulus pattern in a conventional averaging run unless the stimulus duration is a small fraction of the interstimulus interval. (C. II and C. III are not found in VEPs obtained with a stimulus such as pattern reversal where the pattern outlines are continuously present.)

(6) Although a component corresponding to C. I is also found in pattern-offset VEPs, C. II and C. III are found only in pattern-onset responses.

These VEP components appear to reflect the activity of two distinct types of cortical processes: (1) contrast-specific processes located in striate cortex, which show little adaptation to prolonged stimulation; and (2) rapidly adapting processes, more closely related to contours than to contrast, which predominate in extrastriate cortex and whose properties suggest the possible involvement of 'hypercomplex'-type neurones (Hubel & Wiesel, 1965).

C. R. James is supported by M.R.C. Grant G. 973/864.

REFERENCES

HUBEL, D. H. & WIESEL, T. N. (1965). *J. Neurophysiol.* **28**, 1041–1059.
JEFFREYS, D. A. & AXFORD, J. G. (1972). *Expl Brain Res.* **16**, 1–40.

Receptors in the statocyst of squids

BY P. N. DILLY, P. R. STEPHENS and J. Z. YOUNG. *Department of Anatomy, University College London, Gower Street, London WC1E 6BT*

The cavity of the statocyst of decapod cephalopods contains rows of projections, the anticristae (Fig. 1). By limiting the flow of endolymph these may allow increased speed of response and sensitivity by the cupulae of the cristae (Govardovskiĭ, 1971). However, they are covered by a network of nerve fibres originating from hair cells (Fig. 2*a*) and may constitute a third set of receptors, additional to the three maculae and quadripartite crista (Budelman, Barber & West, 1973).

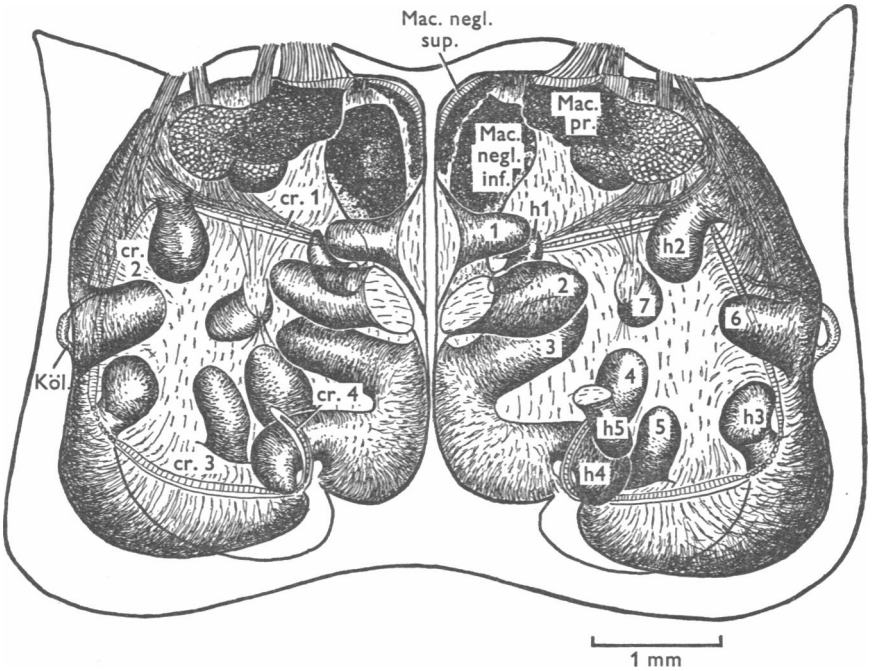

Fig. 1. The statocyst of *Sepia*, from above. 1–7 are straight anticristae and h1–5 are hamuli. The macula princeps carries a large statolith (mac. pr.). The macula neglecta superior is in the vertical sagittal plane, the macula neglecta inferior in the horizontal plane. Köl., Kölliker's canal; cr. 1–4, sections of the crista.

There are two sorts of anticristae – hooks (hamuli) and straight rods. The hooks bend over the crista at each end and where it changes direction. They have hair cells round the base (Fig. 2*b*). The straight anticristae have hair cells whose cilia emerge between the epithelial cells (Fig. 2*c*, *d*). They may respond to movement of the endolymph or bending of the soft

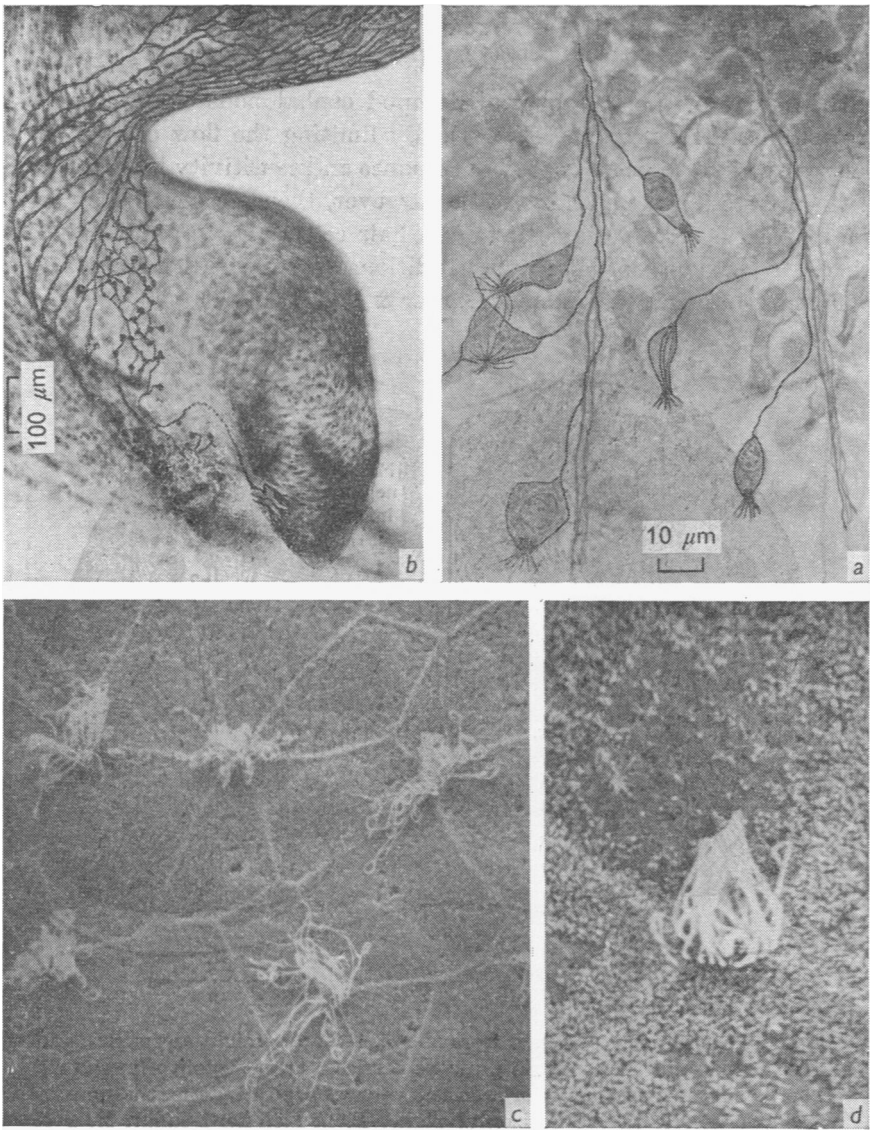

Fig. 2. *a*, Nerve plexus near crista of *Loligo*, with hair cells and their axons touched up (Holmes' silver). *b*, Nerve cells and plexus on one hamulus and one straight anticrista of *Loligo* (methylene blue, touched up). *c*, *d*, Epithelium and projecting hairs of *Sepia* seen with SEM (field widths 42·5 and 9·6 μm).

anticristae when the animal accelerates or decelerates forwards or backwards. Possibly there is also information from deformation of the large bulges in the walls of the sac, which are also covered by nerve fibres.

REFERENCES

BUDELMANN, B. U., BARBER, V. C. & WEST, S. (1973). *J. cell. comp. Physiol.* **85**, 283–290.

GOVARDOVSKIĬ, V. I. (1971). *J. evol. biochem. Physiol.* **7**, 410–416.

The effects of axotomy and Nerve Growth Factor on the neuronal population of the superior cervical ganglion of the mouse

BY B. E. C. BANKS and S. J. WALTER.* *Department of Physiology, University College London, Gower Street, London WC1 6BT*

The injection of large amounts of Nerve Growth Factor (NFG) into neonate mice is known to result in the production of sympathetic ganglia that contain more neurones than are normally found in the adult animal. The excess neurones rapidly disappear when injections of NGF are discontinued (Banks, Charlwood, Edwards, Vernon & Walter, 1975). This demonstrates that NGF is required to maintain the viability of this population of neurones. In addition, it has been reported that sympathetic neurones possess a highly specific mechanism for the retrograde transport of NGF (Hendry, Stockel, Thoenen & Iversen, 1974; Stockel, Paravicini & Thoenen, 1974). This has led to the suggestion that retrograde transport of NGF, produced in the end organ, may play a role in maintaining the integrity of sympathetic neurones resulting from normal development. The present experiments were designed to investigate whether the decrease in the number of neurones after post-ganglionic denervation could be prevented by the administration of large amounts of NGF.

Unilateral section of both major post-ganglionic nerves from the superior cervical ganglia of 14-day-old mice was performed under Halothane. Sham operations were carried out on litter-mates. Half the mice were then injected daily with 20 μg/g body weight per day of 7S NGF, while the controls received saline injections. The mice were killed either 1 or 3 weeks after the operations and the ganglia removed, weighed, sectioned, and stained with cresyl fast violet. The density of neurones in each ganglion was determined by counting the number of nuclei within a given area of each section, chosen at random, and the relative numbers of neurones present in the ganglia were estimated from the products of the ganglion weights and mean cell densities. Under the conditions of this experiment post-ganglionic denervation

* Present address: Department of Physiology, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF.

resulted in a marked decrease in cell number, while, concurrently, NGF increased the number of mature sympathetic neurones. These effects appeared to be independent. Thus the hyperplastic response to NGF does not depend on an intact post-ganglionic innervation.

A second experiment was performed in which the mice were injected with NGF from birth for the 14 days preceding the operation. This resulted in the production of ganglia containing approximately 3·4 times as many neurones as were present in control animals. After axotomy there was a 40% fall in cell number, whereas in mice untreated with NGF axotomy resulted in the disappearance of more than 80% of the neuronal population. Thus NGF appears to be able to maintain the viability of those excess neurones that result from its action even after post-ganglionic denervation. In contrast, the cell death that occurs after axotomy in those neurones that result from normal development appears to be unaffected by NGF. It thus seems probable that the enlarged ganglia obtained from mice given daily injections of NGF may contain two different populations of neurones.

REFERENCES

BANKS, B. E. C., CHARLWOOD, K. A., EDWARDS, D. C., VERNON, C. A. & WALTER, S. J. (1975). *J. Physiol.* **247**, 289–298.

HENDRY, I. A., STOCKEL, K., THOENEN, H. & IVERSEN, L. L. (1974). *Brain Res.* **68**, 103–121.

STOCKEL, K., PARAVICINI, U. & THOENEN, L. L. (1974). *Brain Res.* **76**, 413–421.

Connexions between the cerebellum and the reticular formation in the dogfish *Scyliorhinus canicula*

BY D. H. PAUL and B. L. ROBERTS. *Department of Physiology, Manchester University, M13 9PT, and the Laboratory of the Marine Biological Association, Plymouth PL1 2PB*

The experiments were performed on fish that were initially anaesthetized with tricaine methane sulphonate (0·5 g/l. solution in sea water) and then decerebrated. After recovery of spontaneous respiratory movements, the fish were paralysed by an intravenous dose of curare and artifically respired.

The fish were stimulated peripherally, using natural or electrical stimulation of fins or skin at intensities sufficient to elicit a motor reflex in an unparalysed fish. This procedure is known to modulate the discharges of cerebellar Purkinje cells (Paul & Roberts, 1975). In addition, in some of the fish the region of the cerebellar peduncle and nucleus was stimulated with a bipolar concentric electrode. In the others, the same electrode was used to stimulate the reticular formation.

With peduncular stimulation and micro-electrode recording from the

reticular formation, the following categories of evoked unit discharges were identified:

(*a*) Units invaded antidromically from the cerebellum (latency < 1 msec); some of these units were also driven by the peripheral stimulus (with electrical stimulation, latency 5·6–20 msec). Occasionally a unit was evoked by a peripheral stimulus that was well below threshold intensity for eliciting a reflex in an unparalysed fish.

(*b*) Units excited orthodromically from the cerebellum (latency 3–5 msec) and by peripheral stimulation (latency 11–20 msec). The response to a single shock to the cerebellar peduncle could be greater than that to a train of stimuli applied peripherally.

(*c*) Units evoked from the cerebellum with latencies of 1–1·8 msec; one only responded with a multiple spike discharge. Some of these units also responded to peripheral stimulation.

(*d*) Units driven orthodromically from the cerebellum (latency 3–5 msec) but insensitive to any form of peripheral stimulation (cutaneous, vestibular, lateral line, passive movement).

By stimulating the reticular formation and recording from the peduncle/nuclear region, very short latency antidromic and longer latency, presumably orthodromic field potentials were recorded. Single units were isolated very infrequently; those observed had either a very short (< 1 msec) latency and were classed as antidromic, or a latency of at least 2–3 msec and were classed as orthodromic.

These experiments show the existence of a bidirectional connexion between the cerebellum and the reticular formation. However, it seems unlikely that the Purkinje cells which have their discharge modulated during a reflex movement (Paul & Roberts, 1975) are driven through a reticular path; the latency of the reticular responses to peripheral stimulation was too short in most instances. On the other hand, the results confirm electrophysiologically the conclusion of Ebbesson & Campbell (1973), working on the nurse shark (*Ginglymostoma cirratum*) that a major cerebellar projection terminates on cells of the reticular formation.

This project has been supported in part by a grant to one of us (D.H.P.) from the S.R.C.

REFERENCES

EBBESSON, S. O. E. & CAMPBELL, C. B. G. (1973). *J. comp. Neurol.* **152**, 233–254.
PAUL, D. H. & ROBERTS, B. L. (1975). *J. Physiol.* **244**, 47*P*.

The effect of cholinergic and anticholinergic drugs on rotational behaviour in mice with destruction of one nigrostriatal pathway

By C. D. Marsden, J. Milson, J. D. Parkes, C. Pycock and D. Tarsy.
University Department of Neurology, Institute of Psychiatry and King's College Hospital, London, S.E.5

Clinical and experimental evidence suggests a reciprocal balance between dopaminergic and cholinergic systems in the basal ganglia. The simplest model to explain this relationship is that the dopaminergic nigrostriatal pathway directly inhibits striatal cholinergic neurones (Batholini, Stadler & Lloyd, 1973*a*, *b*). This hypothesis has been tested by examining the effects of cholinergic and anticholinergic drugs on the rotational behaviour produced by apomorphine and amphetamine (Ungerstedt, 1971) in mice with a unilateral destruction of one nigrostriatal dopaminergic pathway. The latter was achieved by direct intrastriatal injection of 6-hydroxydopamine (Von Voigtlander & Moore, 1973). Such mice, tested 5–30 days after surgery, rotate towards the injected side when given amphetamine, due to release of dopamine from the intact nigrostriatal terminals, and away from the injected side when given apomorphine, due, it is believed, to preferential stimulation of the denervated 'supersensitive' striatal dopamine receptors. The hypothesis suggests that this turning behaviour is mediated by inhibition at dopamine receptors on the striatal cholinergic neurones, and that cholinergic drugs might be expected to inhibit, and anticholinergics to potentiate drug-induced turning behaviour.

Physostigmine (200 μg/kg I.P., 30 min beforehand) did inhibit, and scopolamine (1 mg/kg I.P., 30 min beforehand) did potentiate amphetamine-induced (0·5–2 mg/kg I.P.) ipsiversive circling. However, although physostigmine reduced contraversive circling caused by a low dose of apomorphine (0·5 mg/kg I.P.), it had no effect on that caused by a higher dose (2 mg/kg I.P.), and scopolamine did not alter the circling produced by either dose of apomorphine. This suggests that scopolamine was acting only on the side with an intact nigrostriatal pathway, a conclusion supported by the observation that scopolamine (0·5–4 mg/kg I.P.) by itself caused ipsiversive circling, and by the observation that scopolamine-induced circling was abolished by pre-treatment with haloperidol (1 mg/kg I.P., 30 min beforehand) or α-methyl-*p*-tyrosine methyl ester (250 mg/kg I.P., 5 hr beforehand).

These findings suggest that drugs influencing cholinergic neurotransmission, when administered systemically, effect turning behaviour in mice with a unilateral destruction of one nigro-striatal pathway by manipulating the release of dopamine from the remaining intact nigrostriatal system. This is unlikely to be due to the known capacity of certain anticholinergics

to block dopamine re-uptake (Coyle & Snyder, 1969; Horn, Coyle & Snyder, 1971), for while benztropine is over a 1000 times more potent than scopolamine in this respect, it was much less effective than scopolamine in potentiating amphetamine-induced circling, or in causing ipsiversive circling by itself.

The results are compatible with a cholinergic neurone inhibiting the nigrostriatal system, but physiological evidence for such a synapse is not available. Whether it could be in the striatum itself or in the substantia nigra remains to be determined, although biochemical evidence suggests the latter (Bartholini *et al.* 1973*a*; Javoy, Agid & Glowinski, 1973). However, this hypothesis would explain why anticholinergic drugs are less effective than levodopa in the treatment of Parkinson's disease.

REFERENCES

BARTHOLINI, G., STADLER, H. & LLOYD, K. G. (1973*a*). *Adv. Neurol.* **3**, 233–241.
BARTHOLINI, G., STADLER, H. & LLOYD, K. G. (1973*b*). In *Frontiers in Catecholamine Research*, ed. USDIN, E. & SNYDER, S., pp. 741–745. Oxford: Pergamon Press.
COYLE, J. T. & SNYDER, S. H. (1969). *Science, N.Y.* **166**, 899–901.
HORN, A. S., COYLE, J. T. & SNYDER, S. H. (1971). *Molec. Pharmac.* **7**, 66–80.
JAVOY, F., AGID, Y. & GLOWINSKI, J. (1973). In *Frontiers in Catecholamine Research*, ed. USDIN, E. & SNYDER, S., pp. 747–749. Oxford: Pergamon Press.
UNGERSTEDT, U. (1971). *Acta physiol. scand.* suppl. 367, pp. 49–93.
VON VOIGTLANDER, P. F. & MOORE, K. E. (1973). *Neuropharmacology* **12**, 451–462.

The cells of origin of the spinocervical tract

BY A. G. BROWN, C. R. HOUSE, P. K. ROSE* and P. J. SNOW.* *Department of Physiology, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh EH9 1QH*

The spinocervical tract (SCT) takes its origin from neurones in the dorsal horn of the spinal cord (Hongo, Jankowska & Lundberg, 1968; Bryan, Trevino, Coulter & Willis, 1973) some of which can be seen, in Golgi preparations, to have axons running to the dorsolateral funiculus (see Réthelyi & Szentagothai, 1973). The aim of the present experiments was to record from SCT neurones, determine their physiological properties, and then inject Procion dyes into them in order to reconstruct the morphology of the cells so that the physiology and morphology might be examined for correlations. Best results were obtained with Procion Yellow M4R and Procion Scarlet MG.

The experiments were performed on cats (decerebrate, decerebrate-spinal or anaesthetized with chloralose). Full details of the electrophysiological and histological methods have been decribed (Brown, House &

* Post-doctoral Fellow of the Canadian M.R.C.

Hume, 1975). In order to find SCT cells the cord was searched routinely to a depth of 2500 μm, and often to 3000 μm, from the dorsum.

A total of 24 SCT neurones were marked with Procion dyes and of these nineteen were well filled as judged by a comparison with pictures of Golgi stained material. A further fifty-one SCT neurones were recorded extracellularly. SCT cells were impaled at depths between 1200 and 2200 μm from the cord dorsum (350–750 μm from the dorsal border between white and grey matter when measured from histological sections; uncorrected for shrinkage) and maximal extracellular recordings were made between 1100 and 2200 μm.

Some neurones had only dorsally directed dendrites but most also had dendrites extending in horizontal and ventral directions. Neurones with somas lying superficially in the dorsal horn had dorsally directed dendrites which reached the border of the grey matter or to within 200 μm of it, whereas deeply situated cell bodies had dorsally directed dendrites which failed to reach within 200 μm of the dorsal border of the grey matter. The axons of the cells were stained varying amounts (up to a length of 1500 μm) and with one possible exception there were no indications of collateral branching.

No correlations were observed between the physiological type and the morphology of the SCT cells, nor was there any correlation between physiological type and depth of the cell in the spinal cord. There was no correlation between cell soma volume and axonal conduction velocity.

We conclude that the set of neurones which gives rise to SCT axons is not morphologically homogeneous. SCT neurones are situated between 1100 and 2200 μm from the cord dorsum, in general agreement with the results of Hongo *et al.* (1968).

We wish to thank Mr R. B. Hume for excellent technical assistance. This work is supported by a grant to A.G.B. from the M.R.C. The Procion dyes were kindly provided by a gift from I.C.I.

REFERENCES

Brown, A. G., House, C. R. & Hume, R. B. (1975). *J. Physiol.* **244**, 10–12.

Bryan, R. N., Trevino, D. L., Coulter, J. D. & Willis, W. D. (1973). *Expl Brain Res.* **17**, 177–189.

Hongo, T., Jankowska, E. & Lundberg, A. (1968). *J. Physiol.* **199**, 569–592.

Réthelyi, M. & Szentagothai, J. (1973). In *Handbook of Sensory Physiology*, vol. II, *Somatosensory System*, ed. Iggo, A., pp. 207–252. Berlin, Heidelberg, New York: Springer Verlag.

Excitation of frog spinal motoneurones by glycine

By P. R. Adams and D. B. Pixner. *Pharmacology Department, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ*

In the isolated amphibian spinal cord glycine has been reported to produce hyperpolarizing d.c. shifts in the ventral roots, indicative of similar changes at the level of the motoneurone membrane (Tēbecis & Phillis, 1969; Barker & Nicoll, 1973). In both studies, however, glycine produced depolarizing d.c. shifts in a number of preparations.

We have found that in the isolated hemisected frog spinal cord, glycine typically enhanced the evoked ventral root discharge (of motoneurones) to dorsal root or lateral column stimulation and often gave rise to firing in the absence of electrical stimulation despite added magnesium. The amplitude of glycine-induced depolarization was thus sufficient to exceed firing threshold in a number of motoneurones. These consistent and unexpected effects were further explored.

Ventral root d.c. polarization levels were recorded with silver chloride suction electrodes. Intracellular recordings were made from lumbar motoneurones with acetate-filled micro-electrodes. Lumbar ventral root responses were evoked by shocks applied to the ipsilateral corresponding dorsal root or to the ventrolateral surface of the cord to evoke a monosynaptic response (Brookhart & Fadiga, 1960). The motoneurones could be activated non-synaptically either by antidromic ventral root stimulation or directly by passing current through the micro-electrode. The control (oxygenated frog Ringer solution) and drug solutions (glycine, sodium glutamate, strychnine hydrochloride) were applied by superfusion. On a few occasions, glutamate was applied iontophoretically. Glycine action was studied on thirty-nine cord preparations.

Glycine (2×10^{-3} M) produced depolarizing d.c. shifts in the ventral roots, accompanied by reductions of motoneurone membrane potential and resistance, even in the presence of 5 mM-$MgCl_2$. When subthreshold depolarization was produced (either synaptically or directly), the additional depolarization produced by glycine was sufficient to exceed firing threshold for the cell. When antidromic stimulation was ineffective in initiating a cell response, the subsequent glycine-induced depolarization usually precipitated somadendritic invasion of the motoneurone. These actions of glycine resembled those of glutamate (approx. 6×10^{-4} M). Our findings with strychnine accord with those of Tēbecis & Phillis (1969), who found no modification of the depolarizing response to glycine.

The ability of strychnine to enhance the evoked synaptic potential indicates that post-synaptic inhibition had prevailed during these evperiments. It is unlikely then that glycine had become excitatory,

consequent to prior reversal of the transmembrane chloride concentration gradient. These observations suggest that glycine exerts primarily excitant actions in this preparation through a sodium-dependent (strychnine insensitive) mechanism, distinct from that described in the cat (Werman, Davidoff & Aprison, 1968).

REFERENCES

BARKER, J. L. & NICHOLL, R. A. (1973). *J. Physiol.* **228**, 259–277.
BROOKHART, J. M. & FADIGA, E. (1960). *J. Physiol.* **150**, 633–655.
TĒBECIS, A. K. & PHILLIS, J. W. (1969). *Comp. Biochem. Physiol.* **28**, 1303–1315.
WERMAN, R., DAVIDOFF, R. A. & APRISON, M. H. (1968). *J. Neurophysiol.* **31**, 81–95.

The influence of hypercapnia and hypoxia on the activity recorded from intestinal sympathetic nerves in the rabbit with cut sinus nerves

BY E. A. BOWER. *Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG*

The sympathetic response to carotid body stimulation is well established (Gernandt, Liljestrand & Zotterman, 1946), but severe hypoxia can also increase sympathetic activity by central excitation (Alexander, 1945; Bernthal & Woodcock, 1951). The present experiments were designed to establish more exactly the arterial P_{O_2} at which this effect starts, and to test whether it is potentiated by hypercapnia.

The sinus, aortic, vagus and cervical sympathetic nerves were cut in rabbits anaesthetized with urethane (1·5 g/kg). After the administration of gallamine, positive pressure ventilation was used so that arterial P_{O_2} and P_{CO_2} could be varied independently by use of different gas mixtures and by alterations in respiratory pump stroke volume. The control P_{CO_2} was between 31 and 38 mmHg. Action potentials recorded from the central ends of fine intestinal nerves were counted by a ratemeter; respiratory movements, arterial and venous pressures were also measured and 0·5 ml. arterial blood samples were taken for estimation of P_{O_2}, P_{CO_2} and pH. The activity in the nerve was abolished if tetraethylammonium or hexamethonium was injected intravenously.

Hypercapnia did not alter the discharge until the P_{CO_2} exceeded 45 mmHg; thereafter the frequency rose in proportion and was doubled at about P_{CO_2} 75 mmHg, the pH having fallen by about 0·25 units. At the termination of hypercapnia the discharge returned gradually to the control level. A comparable fall in pH induced by slow I.V. infusion of 0·2 M-HCl gave only a small transient rise in discharge frequency.

Hypoxia had little effect on the discharge unless the P_{O_2} fell to 25 mmHg.

Below this level the frequency rose sharply and had doubled at P_{O_2} 15–20 mmHg. The characteristic response to hypoxia was a rapid increase in frequency at 15–30 sec followed by wide fluctuations; after the termination of hypoxia the discharge fell abruptly and sometimes stopped completely for up to 15 sec before returning slowly to control values. The action of hypoxia could not be attributed to the fall in arterial pressure which usually occurred, since an equivalent or greater fall in pressure induced by amyl nitrite or haemorrhage had no effect on the discharge, unless the pressure fell below 20–25 mmHg, at which level the frequency increased rapidly.

During asphyxia the effects resembled those of hypercapnia provided the P_{O_2} was above 30 mmHg, but at P_{O_2} 25–30 and P_{CO_2} 55–65 the frequency rose sharply and the discharge developed the pattern characteristic of hypoxia. The frequency was increased by about $3\frac{1}{2}$ times at P_{O_2} 20 mmHg. In any one rabbit, the discharge in severe asphyxia was more intense than in hypoxia at the same P_{O_2}, and in some cases was greater than a simple summation of the hypoxic and hypercapnic excitation.

REFERENCES

ALEXANDER, R. S. (1945). *Am. J. Physiol.* **143**, 698–708.

BERNTHAL, T. & WOODCOCK, C. C. JR. (1951). *Am. J. Physiol.* **166**, 45–54.

GERNANDT, B., LILJESTRAND, G. & ZOTTERMAN, Y. (1946). *Acta physiol. scand.* **11**, 230–247.