photomultiplier. Since most of the pigment molecules have their chromophore aligned perpendicular to the rod axis, it is advantageous to make measurements with light polarized in this plane. Maximum absorbance values of between 0·4 and 0·7 can then be obtained for pigment in an unbleached axolotl retina, whereas with a conventional spectrophotometric technique, where the light is made to pass axially through the receptors, the values rarely exceed 0·15.

The use of plane polarized light also allows investigation of the chromophore orientation in the slowly disappearing products of bleaching. This remains the same as in the parent molecule but vitamin A, the final product, is aligned along the rod axis. Hence difference spectra for the parent pigment obtained with light polarized perpendicular to the rod axis will be little contaminated by vitamin A.

This work was supported by the Medical Research Council.

### A method for perfusing the anterior chamber of the rabbit eye

By D. F. Cole. *Department of Experimental Ophthalmology, Institute of Ophthalmology, Judd Street, London WC1H 9QS*

### Hind-gut function of domestic fowl

By A. R. Tindall. *Box 977, 9001 Tromso, Norway*

## COMMUNICATIONS

### Behaviour of short and long latency components of the stretch reflex in human muscle

By C. D. Marsden, P. A. Merton and H. B. Morton. *The National Hospital, Queen Square, London, W.C.1*

In the long flexors of the thumb and the big toe and the muscles of the jaw the latency of the stretch reflex and of other manifestations of servo action is about double the time round the segmental reflex arc, as evidenced by tendon jerk latencies. Such long latency stretch reflexes are suspected to use a trans-cortical rather than a spinal pathway (Phillips, 1969; Marsden, Merton & Morton, 1972, 1973).

In biceps (Hammond, 1960) and in shoulder girdle muscles we find both spinal latency and long latency components in the stretch reflex, and in the responses to release and probably to halt. The long latency component (40 msec in infraspinatus) has been seen virtually uncontaminated in a healthy undergraduate in whom tendon jerks were clinically absent

in all muscles except biceps, where they could be obtained only with intense reinforcement. Electromyographically a tendon jerk could also be seen (at 13 msec) with reinforcement in contracting infraspinatus, but reinforcement did not change the long latency component.

A dissociation of the long latency and spinal components had been made earlier in the hand, when it was discovered that anaesthesia of the hand produced by a cuff distal to the muscle greatly depressed the stretch reflex in the long flexor of the thumb, without affecting the finger jerks. This was provisionally interpreted to mean that signals from the skin of the thumb (or possibly from the joints) have to cooperate at cortical level with those from muscle spindles to permit stretch reflexes to occur.

For infraspinatus, whose function is more postural than exploratory, it was not unexpected to find that neither component of the stretch reflex was affected by profound anaesthesia of the whole arm from a cuff inflated as high on the upper arm as possible. More surprisingly, it turns out that stretch reflexes in the long flexor of the big toe are also unaffected by peripheral anaesthesia. This was produced by a cuff round the instep, left on for well over an hour. Would the result have been different if our subjects had been trained to write with their toes? On a lower plane, the experiment shows that the long latency human stretch reflexes we deal with, at any rate those in the toe, are due to muscle receptors, a question somewhat in doubt after the thumb results with anaesthesia.

This work was assisted by a grant from the Bethlem Royal and Maudsley Hospitals Research Fund.

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## Sodium entry in junctional region of rat muscle

BY R. CREESE and L. MITCHELL. *Department of Physiology, St Mary's Hospital Medical School, London W2 1PG*

The action of depolarizing compounds on muscle end-plates has been interpreted in terms of the movement of sodium ions or other cations (Katz & Miledi, 1972). Attempts to detect the influx of labelled sodium at the end-plate meet a number of experimental difficulties, and the following method was used.

A strip of diaphragm was immersed for 15–30 sec in saline (Creese & Northover, 1961) which contained $^{24}Na$ plus depolarizing drug. After

exposure the muscle was passed through a succession of tubes of inactive saline for a standard time of 6 min so that the labelled sodium in the extracellular fluid could be washed out. The muscle was removed and frozen on solid carbon dioxide, and the band of end-plates was located (England, 1970). The muscle was sliced at intervals of 1 mm from tendon to rib and the tissues were weighed, dissolved and counted by liquid scintillation. Tetrodotoxin (0·1 $\mu$M) was added to the saline (Colquhoun, Rang & Ritchie, 1974), and it was found in parallel experiments that the depolarization could be recorded by internal electrodes without the production of action potentials.

When the labelled sodium content, following exposure to carbachol (100 $\mu$M) for 15 sec, was plotted as a function of distance along the fibre, a peak was obtained in the slice which contained the band of end-plates. The values at the ends of the muscle when averaged were similar to the control muscle. The uptake of labelled sodium in control muscles without depolarizing drug was equivalent to 87 p-mole $mg^{-1}$ (median of 10, range 68–147), and these results are comparable with previous findings (Creese, 1968). Exposure to carbachol for longer times up to 1 min also gave peaks of radioactivity in the end-plate region.

The method allows comparison between the influx at the junctional region and that at the ends of the muscle. The uptake in the central slices, less the value assigned to that at the ends of the muscle, was taken as a measure of the excess sodium which had entered as a result of the action of the depolarizing drug. The peak labelled sodium content is approximately twice the value at the end, and for decamethonium (100 $\mu$M for 30 sec and wash for 6 min) the ratio of peak to end was 1·84 (median of 12, range 1·46–2·03).

The extracellular sodium has to be removed to demonstrate uptake at the junctional region, but during this time the labelled sodium which has entered is depleted both by exchange with inactive saline and also by diffusion along the fibre from the site of entry. Extension of the wash beyond 6 min produced further loss of radioactivity and also a widening of the bell-shaped curves which could be fitted to the histograms, as expected if diffusion takes place along the fibres.

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## Ionic mechanisms in *Aplysia* neurones as affected by intracellular actions of phospholipases A and C

By D. H. Hinzen* and L. Tauc. *Laboratoire de Neurobiologie Cellulaire du CNRS, 91190 Gif-sur-Yvette, France*

The selective enzymatic alteration of neuronal membranes by internally applied pure phospholipase A (PLA) and C (PLC) might provide a useful tool for the study of the molecular structure underlying ionic conductances. Mammalian PLA (gift of G.H. de Haas (de Haas, Postema, Nieuwenhuizen & van Deenen 1968)), and bee venom PLA (gift of C. A. Vernon (Shipolini, Callewaert, Cotrell, Doonan, Vernon & Banks 1971)), both exhibiting activities of the $A_2$ type; as well as PLC from *Bacillus cereus* (gift of R. F. A. Zwaal (Zwaal, Roelobsen, Comfurius & van Deenen 1971)), and PLC from *Clostridium welchii* (own preparation, unpublished), were used. All enzymes were dissolved at 5% concentration in a glycerol–2·5 M-KCl (9:1) solution. A volume equal to 1% of that of the cell body was injected intracellularly as described by Stinnakre & Tauc (1973). The medial cells (Kehoe, 1972) in the pleural ganglion *of Aplysia californica* were studied using voltage and current clamps.

Bee venom PLA produced a rapid decrease of membrane potential and resistance. Voltage clamping revealed a marked depression of peak transient current with little or no effect on the late outward current. Mammalian PLA was found ineffective in changing either the resting or active membrane properties. PLC from *Bacillus cereus* led to an initial slight hyperpolarization and often to a moderate increase in resting membrane resistance. This was followed by a strong hyperpolarization, and a fall in membrane resistance to one tenth of the original value. The Na and Ca excitable membrane components were only little affected, whereas the late K current was increased. Neurones injected with *Cl. welchii* PLC manifested a several-fold rise in resting membrane resistance, as well as a tendency to slight hyperpolarization.

These enzyme actions followed the intracellular application with a latency of usually 5–30 min depending upon dose. All enzymes were ineffective when externally applied.

Tentative conclusions are as follows. (1) Bee venom PLA increases the Na permeability in the resting membrane and depresses that in the active membrane. (2) *Cl. welchii* PLC reduces the Na permeability of the resting membrane. (3) *Bacillus cereus* PLC augments in the later step the resting and active K permeability. (4) Pancreatic PLA is without effect on this membrane at comparable concentrations. (5) It is not clear what causes

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the differing actions of enzymes of the same type, but it might be reasonable to extend the concept of special lipid–protein interaction and localization, deduced so far from studies in membrane model systems (Verger, Mieras & de Haas, 1973) and red cells (Zwaal, Roelofsen & Colley, 1973), to excitable cell membranes in their actual micro-environmental conditions. This should help to link the membrane phospholipid orientation to electrogenesis.

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## Abnormal cardiovascular reflexes in subjects with autonomic neuropathy

BY T. BENNETT, D. EVANS, J. R. HAMPTON and D. J. HOSKING. *Department of Physiology, Medical School, Nottingham University, and Department of Medicine, General Hospital, Nottingham*

In man, the normal cardiovascular reflex response to apnoea accompanied by immersion of the face in water is bradycardia and peripheral vasoconstriction (Hiestad, Abboud & Eckstein, 1968). We have studied the cardiovascular responses to this manoeuvre in two groups of diabetic subjects. The five subjects forming the control group showed marked sinus arrhythmia (Wheeler & Watkins, 1973), normal responses to Valsava's manoeuvre (Johnson & Spalding, 1974, and to elevation of arterial blood pressure (Smyth, Sleight & Pickering, 1969). Five diabetics, matched for age with the members of the control group, showed little or no sinus arrhythmia, a loss of cardiac vagal reflexes, and signs of postural hypotension; these subjects were, therefore, judged to have autonomic neuropathy.

Apnoea alone caused no significant change in cardiovascular parameters in either group of subjects. In the control subjects, apnoeic face immersion caused a significant bradycardia, fall in forearm blood flow, increase in

mean blood pressure and increase in forearm vascular resistance. In the subjects with autonomic neuropathy, there was a significant tachycardia, increase in forearm blood flow, and fall in forearm vascular resistance; there was no significant change in mean blood pressure.

In both groups of subjects, mental stress caused a significant tachycardia and increase in forearm blood flow. Such responses appear to be due to activation of sympathetic cardioaccelerator mechanisms (Taylor & Meeran, 1973) and sympathetic vasodilator nerves to blood vessels supplying forearm muscles (Blair, Glover, Greenfield & Roddie, 1959).

TABLE 1

| | Control subjects | | Subjects with autonomic neuropathy | |
|---|---|---|---|---|
| | Rest | Apnoeic face immersion | Rest | Apnoeic face immersion |
| Heart rate (beats $min^{-1}$) | 90·6 ± 2·9 | *76·8 ± 6·9 | 96·0 ± 2·9 | *102·2 ± 7·1 |
| Forearm blood flow (ml. 100 $ml.^{-1}$ $min^{-1}$) | 3·€2 ± 0·15 | *2·48 ± 0·37 | 2·30 ± 0·1 | *4·5 ± 0·6 |
| Mean blood pressure (mmHg) | 99·2 ± 1·7 | *107·6 ± 1·8 | 96·9 ± 2·8 | 93·2 ± 7·5 |
| Forearm vascular resistance (arbitrary units) | 28·6 ± 1·4 | *47·6 ± 7·3 | 44·1 ± 2·2 | *22·2 ± 2·7 |

Values are means ± S.E. $n = 5$.

* Significant ($P < 0·05$) differences between values for rest and for apnoeic face immersion.

The similarity in the responses to apnoeic face immersion and mental stress, in the subjects with autonomic neuropathy, suggests that the same mechanisms are being activated under both conditions. This corroborates the suggestion that stimuli which cause bradycardia and vasoconstriction can cause tachycardia and vasodilatation when the normal mechanisms are inoperative (Abboud & Eckstein, 1966).

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## The unsteady tidal air level and air trapping

By H. Herxheimer. *Department of Pharmacology, University College, Gower Street, London, W.C.1*

After a deep inspiration, the spirometer tracing normally returns to tidal air level in one, uninterrupted, smooth movement. In a few subjects, however, this return occurs in 3 or 4 small steps. The current text-books of respiratory physiology (Comroe *et al.* 1962; Cotes, 1968) describe this as a sign of air trapping. Comroe, however, mentions that the same phenomenon may occur after deep expiration, when the tidal air level may also be resumed in small steps. This second observation cannot be due to air trapping, because, during inspiration, no air can be trapped in the lungs, unless one wants to make the unlikely assumption that, during deep inspiration, the lung unfolds stepwise. This never happens. The explanation of the stepwise return to tidal level as air trapping is therefore open to doubt.

It may be due to the inability of the subject to maintain a steady tidal air level. Fig. 1 shows the stepwise return to tidal level from expiration in two instances. In addition, after one deep inspiration, followed by breathholding for 55 sec, the tidal air does not return to its previous level but stays above it, until a new deep expiration causes it to resume the previous level. This occurs also without breathholding. Sometimes, the tidal level remains elevated after each deep inspiration and then requires 2–3 min for its return to the previous level; or it may stay, after a deep expiration, at a lower level. All these variations may occur in asthmatics or in normal subjects with irregular respiration. Air trapping is an unlikely explanation for them.

In contrast, air trapping can be assumed if heavy expiratory pressure causes bronchoconstriction, as happens when forced vital capacity is recorded from maximum inspiration point. This may occur in asthmatics and bronchitics, in whom the bronchial muscle is particularly sensitive to stimulation, and causes narrowing or closure of bronchi or bronchioli. The pressure exerted is considerable (Rahn, Otis, Chadwick & Fenn, 1946; Mills, 1950) and the bronchial constriction so strong that the subject often cannot exhale further than the expiratory tidal level. With exhalation starting from tidal level, the pressure stimulus would be much less, and he could empty his expiratory reserve (the trapped amount) quite normally. For this phenomenon, air trapping seems the suitable term.

It is concluded that the stepwise return to tidal air level should not be termed 'air trapping'. It is probably caused by the inability of the subject to maintain a steady tidal air level.

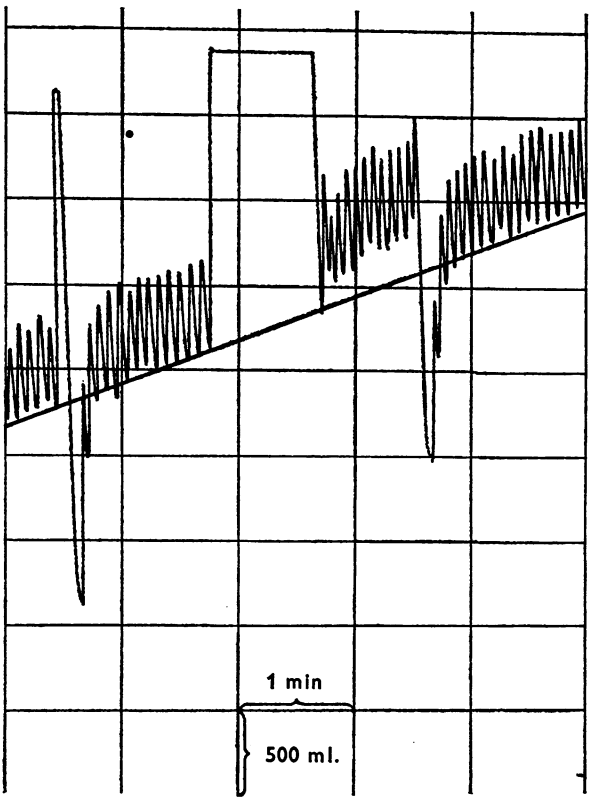


Fig. 1. Spirometric tracing of 2 deep expirations and one deep inspiration. After the latter, breath is held for 55 sec. Note the stepwise return from expiration and the raised tidal level after inspiration. The upward slope of the tracing is due to the $CO_2$ absorption.

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## The action of inhibitors of anion transfer on potassium and calcium movements in metabolically depleted human red cells

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Red cells depleted of metabolic substrates by incubation with iodoacetate take up calcium from Ringer solution and become leaky to potassium but not to sodium (see Romero & Whittam, 1971; Blum & Hoffman, 1971). Under these conditions tests were made to see whether potassium and calcium movements were limited by reducing the rate of anion transfer across the membrane, in a way similar to that in which anion permeability restricts cation loss induced by ionophores in red cells (Hunter, 1971).

Anion permeability was decreased by SITS (4-acetamido-4′-isothiocyano-2,2′-disulphonic stilbene) (Cabantchik & Rothstein, 1972), persantin (dipyridamole) and phloretin (Gerlach, Deuticke & Duhm 1964). Net changes in cell cations (in $\mu$mole/ml. cells) were measured over 2 hr in a Ringer containing iodoacetate (0·5 mM) and calcium (10 mM). Potassium loss was markedly reduced by SITS (0·1 mM) and persantin (0·1 mM) (from $23{\cdot}5 \pm 4{\cdot}3$ to $6{\cdot}3 \pm 3{\cdot}2$ and 0·3 (mean and S.E.M. of 14, 7, 3 experiments respectively)) but calcium uptake was not affected ($1{\cdot}04 \pm 0{\cdot}16$; $1{\cdot}34 \pm 0{\cdot}63$ and $0{\cdot}67 \pm 0{\cdot}09$ respectively). Phloretin (0·9 mM), however, increased potassium loss (to $42{\cdot}2 \pm 5{\cdot}8$; 9 experiments) but reduced calcium uptake (to 0·56; 3 experiments). Potassium leak induced by preloading cells with calcium was inhibited by adding SITS or persantin after 1 hr (with no effect on further calcium uptake) but was again increased by phloretin which prevented any subsequent calcium uptake.

Unidirectional potassium fluxes were measured by tracer. Total influx (in $\mu$equiv. K/ml. cells.hr) with 10 mM external potassium was increased from $9{\cdot}3 \pm 2{\cdot}8$ (8 experiments) to $17{\cdot}4 \pm 4{\cdot}3$ (3 experiments) by addition of SITS but was reduced to $3{\cdot}5 \pm 0{\cdot}8$ (5 experiments) by phloretin. The small ouabain-sensitive component was similar under all conditions, being $1{\cdot}1 \pm 0{\cdot}4$, $1{\cdot}6 \pm 0{\cdot}2$ and $0{\cdot}4 \pm 0{\cdot}1$ respectively. Efflux (expressed as a rate constant in $hr^{-1}$) was reduced from $0{\cdot}50 \pm 0{\cdot}07$ (7 experiments) to $0{\cdot}32 \pm 0{\cdot}02$ by SITS but was not affected by phloretin ($0{\cdot}43 \pm 0{\cdot}08$; 5 experiments).

Influx was increased by 0·31 $\mu$equiv. K/ml.cells.hr per mM K on raising potassium concentration from 1 to 100 mM and was always greater (1·2–1·8 times) with SITS, in agreement with the findings of Hoffman & Knauf (1973). Efflux did not vary appreciably over the same concentration range but was always less with SITS (61–73 % of control). This is in keeping with a smaller net loss of potassium at higher potassium concentrations, which was transformed to a gain by SITS.

The results suggest that limiting the rate of anion transfer with SITS or persantin can reduce potassium loss from cells with raised intracellular calcium levels. On the other hand the decrease in calcium uptake and increase in potassium loss with phloretin may be related to a potentiation of its rather less specific action on anion compared to cation permeability.

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## Resealed ghosts used to study the effect of intracellular calcium ions on the potassium permeability of human red cell membranes

BY T. J. B. SIMONS. *Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG*

Intracellular Ca ions are known to increase the K permeability of human red cells (Blum & Hoffman, 1972) and snail neurones (Meech, 1974), amongst other cells. Nearly all studies with red cells have utilized Ca entry into metabolically depleted cells to induce an increased K permeability, but their results can be hard to interpret because of rapidly changing conditions. The aim of the experiments described here was to make resealed ghosts containing solutions of known, buffered Ca concentration, to discover the optimum conditions for observing an increased K permeability, and then to use those conditions to investigate its properties in an unambiguous fashion.

Fig. 1 shows the effect of incorporated Ca-buffers on the efflux of Na and K ions from resealed ghosts under equilibrium conditions. All the experiments used 4-day-old ACD blood, and the ghosts were prepared and used as previously described (Simons, 1974). The results show that K movements begin to increase at about 0·1 $\mu$M-$Ca^{2+}$, reach a maximum at about 3 $\mu$M, but decline as $Ca^{2+}$ is increased further; while Na movements are unaffected by the $Ca^{2+}$ concentration. Intracellular $Mg^{2+}$ (2 mM) strongly inhibits the increased K permeability at low $Ca^{2+}$ concentrations.

Experiments with resealed ghosts containing an optimum $Ca^{2+}$ concentration showed that only K, Rb and Cs ions had a high efflux rate, while other monovalent cations appeared to inhibit K movements. Their relative

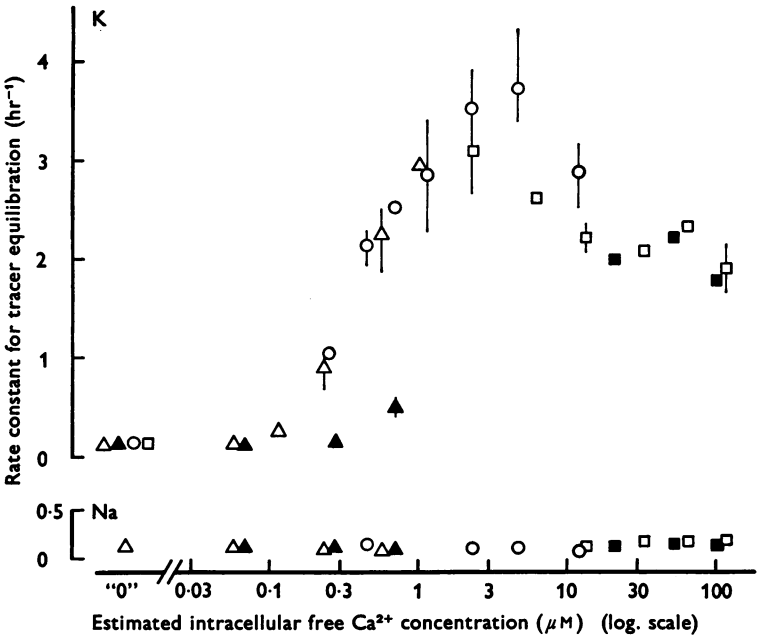


Fig. 1. The effect of intracellular Ca ions on the permeability of red cell membranes to K and Na ions. Resealed ghosts were prepared after lysing 24-hr starved red cells in 15 volumes of a Ca-buffered solution containing $^{22}Na$ and/or $^{42}K$, but no ATP. Rates of tracer efflux, into a Ca-free medium of osmotic pressure 200 ideal m-osmole/l. and ionic strength about 0·1, were measured at 37 °C and pH 7·1 (2 mM phosphate buffer) and an haematocrit of about 4%. Monovalent cations were at equilibrium during flux measurements, and were always sufficient for full activation of the effect (K was usually 90–100 mM). Each point is the average result from up to 6 separate experiments, and the vertical lines indicate the extreme range of the results. Different symbols refer to the anions used as Ca-buffers (total concentration 3 mM): (△, ▲) = EGTA; (○) = HEDTA ((*N*-hydroxyethyl)ethylenediamine-triacetic acid); (□, ■) = citrate. Open symbols (△, ○, □) indicate that no Mg was added (these ghosts actually contained about 0·15 mM total Mg), and closed symbols (▲, ■) that 2–2·5 mM-Mg was present (estimated free $Mg^{2+}$ = 1·5–2 mM). The intracellular free $Ca^{2+}$ concentrations were calculated on the assumption that the ghosts had the same composition as the lysing solutions, which is probably a good approximation. Chemical analysis showed that the ghosts did not lose Ca, Mg or the anionic buffers. The apparent log stability constants used in the calculations, which have been corrected to pH 7·1 and 37° C, were as follows: EGTA: $K'_{Ca}$ = 6·94, $K'_{Mg}$ = 2·97; HEDTA: $K'_{Ca}$ = 5·66; Citrate: $K'_{Ca}$ = 4·15; $K'_{Mg}$ = 2·55.

effectiveness was Li ≃ Na > Choline ≃ TEA. With K concentrations in the 10–100 mM range, Na and choline only affected K movements when present internally. This inhibitory effect was not due to a change in the affinity for Ca of the K permeability mechanism.

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## Excitatory and inhibitory effects of angiotensin and vasopressin on vascular smooth muscle

BY K. GOLENHOFEN and A. H. WESTON.* *Department of Physiology, University of Marburg/Lahn, West Germany*

Tension development was recorded in isolated portal vein and in helical strips of aorta from rabbit, guinea-pig and rat and in helical strips of rabbit ear artery. Electrical activity (intracellular and extracellular) was also recorded in guinea-pig portal vein.

In arteries, angiotensin and vasopressin produced only excitatory responses; vasopressin had no effect on rabbit and guinea-pig aorta. In portal vein, both excitatory and inhibitory effects were seen. Angiotensin ($10^{-8}$–$10^{-5}$ g/ml.) increased spontaneous mechanical activity in rabbit and rat portal vein whilst in the guinea-pig, low concentrations ($5 \times 10^{-8}$–$10^{-6}$ g/ml.) inhibited spontaneous mechanical activity; higher concentrations ($10^{-6}$–$10^{-4}$ g/ml.) produced initial excitation followed by inhibition or, occasionally, an excitatory response alone. Vasopressin ($5 \times 10^{-3}$–$10^{-1}$ i.u./ml.) produced inhibition in both guinea-pig and rabbit portal vein; in the rat, low concentrations ($5 \times 10^{-3}$–$5 \times 10^{-2}$ i.u./ml.) produced excitation whilst higher concentrations (up to $5 \times 10^{-1}$ i.u./ml.) inhibited mechanical activity (Voth, Schipp, Agsten, Schürmann, Kohlhardt & Dudek, 1969).

In extracellular electrical recordings, angiotensin and vasopressin reduced spike discharges. Intracellular recordings (Fig. 1) showed that spike suppression during angiotensin inhibition was accompanied by a hyperpolarization (mean 3 mV, $n = 9$) which was smaller than during comparable isoprenaline inhibition (mean 10 mV, $n = 10$). Vasopressin produced similar effects to angiotensin (mean hyperpolarization 4 mV,

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$n$ = 11). Tachyphylaxis to angiotensin was observed in arterial tissues (Bohr, 1974) but not in portal vein.

A differentiation of phasic and tonic activation mechanisms has recently been reported (Golenhofen, Hermstein & Lammel, 1973; Golenhofen & Hermstein, 1974). Thus, the ability of angiotensin to stimulate tonic

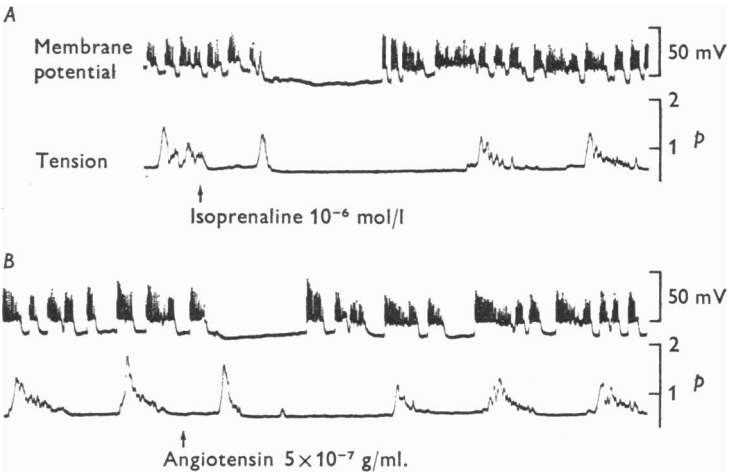


Fig. 1. Effects of (*A*) isoprenaline and (*B*) angiotensin on membrane potential and tension development in guinea-pig portal vein. Sections of a continuous intracellular recording in the same cell.

activity in arterial smooth muscle and simultaneously to inhibit phasically active vessels such as portal vein may further indicate that a differentiated control of phasic and tonic activities is of physiological significance.

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## The participation of muscarinic receptors in the physiological excitation of spinal Renshaw cells

By H. L. Haas* and R. W. Ryall. *Department of Pharmacology, University of Cambridge*

Acetylcholine excites many neurones throughout the central nervous system. At supraspinal levels the effects are usually blocked by the administration of atropine and are therefore considered to be mediated via muscarinic receptors. In the spinal cord the best established site for cholinergic transmission is at the synaptic junctions formed by the terminals of motor axon collaterals ending on Renshaw cells. At this site the major part of the action of acetylcholine administered by micro-electrophoresis seems to involve nicotinic receptors. In addition, the intense early discharge evoked by a synchronous ventral root volley also seems to involve nicotinic receptors (Curtis & Ryall, 1966*a*, *b*). It occurred to us that the much weaker muscarinic actions of acetylcholine, which seemed to be concerned in the mediation of the relatively small late discharge evoked by a synchronous antidromic ventral root volley might assume more importance when synapses are activated more physiologically, by 'natural' stimulation of receptors. Experiments in non-anaesthetized, intercollicularly decerebrate cats, in anaesthetized cats with intact spinal cords or after complete spinal transection in the upper lumbar region revealed that Renshaw cells were excited to varying degrees by stretch of Achilles tendon and that these responses could be markedly reduced or even abolished by relatively small amounts of atropine sulphate (0·1–0·5 mg/kg intravenously) but not by mecamylamine administered either intravenously or micro-electrophoretically. If the receptors blocked by atropine are the muscarinic receptors previously demonstrated on Renshaw cells then it is postulated that the cholinergic pathways to the Renshaw cell may under some conditions utilize nicotinic post-synaptic receptors and under other conditions utilize muscarinic post-synaptic receptors. It is even possible, although we cannot prove it, that the two receptors are both present at the same synaptic junction. It is of some interest that the nicotinic receptor, which is generally that which receives most attention, is most easily revealed by the grossly unphysiological technique of electrically stimulating the central ends of ventral roots whereas less well known muscarinic receptors appear to be more important when the Renshaw cell is excited by more physiological activation of peripheral receptors, as in the present study.

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## Depolarization of sympathetic ganglion cells mediated through the release of γ-aminobutyric acid (GABA) from adjacent glial cells

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Sympathetic ganglia normally contain very low concentrations of GABA (0·1–0·3 mM: N. G. Bowery & G. G. S. Collins, unpublished measurements). However, *oligodendroglial* cells in the ganglion readily accumulate exogenously presented GABA (Bowery & Brown, 1972; Young, Brown, Kelly & Schon, 1973). Also, sympathetic *neurones* are depolarized by GABA (De Groat, 1970; Adams & Brown, 1973; Bowery & Brown, 1974). We consider here whether, following glial accumulation, GABA could be released from the glial cells at a sufficient rate to depolarize the adjacent neurones.

We have tested this by measuring the effect of loading ganglia with GABA on the depolarizing action of 3-amino-*n*-butyric acid (β-aminobutyric acid, BABA). This compound is a very weak GABA-receptor agonist (Bowery & Brown, 1974) but is a substrate for the GABA carrier and thereby stimulates the efflux of GABA from the glial cells (Bowery, 1974).

When the ganglion was loaded with GABA by exposure to 1 mM-GABA for 60 min the depolarizing action of BABA was clearly potentiated (Fig. 1). This was most pronounced when metabolism of GABA was prevented with 10 μM amino-oxyacetic acid (AOAA), and corresponded to the effect of an additional 3–6 μM GABA. In the absence of AOAA, potentiation was equivalent to 1–2 μM additional GABA. This reflects the difference in the intracellular GABA concentrations attained under these conditions ($+$AOAA, $1{\cdot}32 \pm 0{\cdot}106$ mM; $-$AOAA, $0{\cdot}33 \pm 0{\cdot}045$ mM; $n = 4$). Potentiation was not due to a changed sensitivity of GABA-receptors since neither GABA nor 3-aminopropanesulphonic acid – a receptor-agonist with low carrier-affinity – were potentiated.

We conclude that release of GABA from the loaded glial cells accounts for the increased response to BABA. In brain endogenous GABA concentrations may exceed those attained artificially in ganglia by loading. Thus, substrates for GABA carriers in the brain might release sufficient

GABA from glial cells or nerve terminals to produce indirect GABA-like actions on central neurones.

This investigation was supported by a grant from the Medical Research Council.

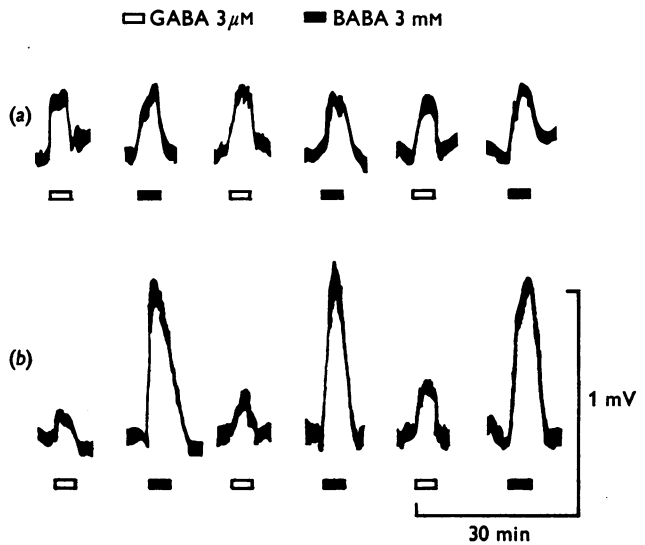


Fig. 1. Depolarizations of the isolated rat superior cervical ganglion produced by just-suprathreshold concentrations of GABA and BABA (*a*) before and (*b*) 1 hr after perfusing the ganglion with 1 mM GABA in the presence of 10 μM amino-oxyacetic acid. The ganglion was superfused with Krebs solution at 25° C. Depolarization was recorded with surface electrodes (Brown & Marsh, this meeting). Agonists were added for 4 min at 25 min intervals.

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## Intersegmental reflex excitation of leg muscles and myochordotonal efferents in decapod Crustacea

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The small 'accessory flexor muscle' (AF) and associated multicellular proprioceptors in crustacean limbs together function quite similarly to

the vertebrate muscle spindle (Evoy & Cohen, 1971). One excitatory and one inhibitory motoneurone provide efferent regulation (Angaut-Petit, Clarac & Vedel, 1974). This single myochordotonal organ, like the other limb chordotonal organs (multicellular stretch receptors lacking efferent control), evokes 'resistance reflexes' analogous to mammalian stretch reflexes (Bush, 1965).

We have now demonstrated an intersegmental proprioceptive reflex, from the single chordotonal organ (CB) of the coxo-basal joint to both main muscles operating the mero-carpopodite joint *and* to AF. Extracellular e.m.g.s were obtained in intact crayfish and rock-lobsters, and intracellular recordings from the partially exposed AF in shore crabs.

The AF excitor commonly showed a low frequency background discharge, dependent partly upon mero-carpopodite joint position, and often

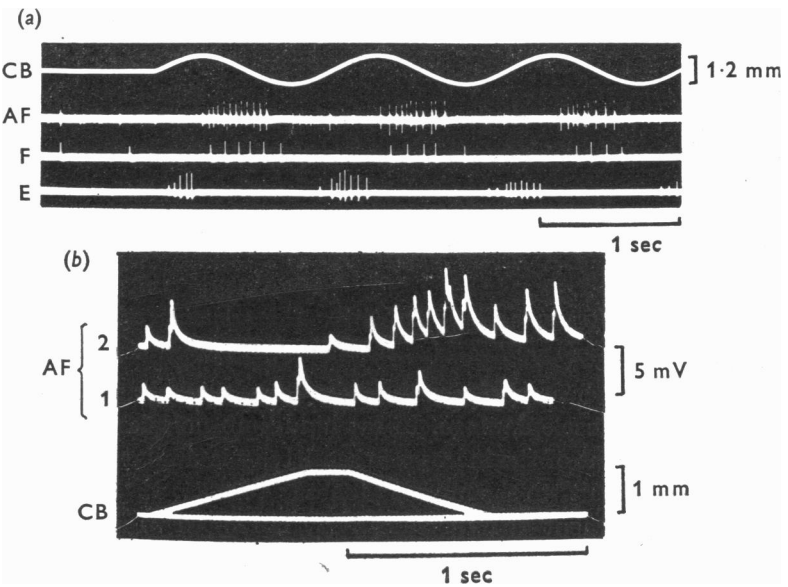


Fig. 1. Reflex responses to applied length changes of the coxo-basal chordotonal organ, CB (stretch upwards), recorded from the accessory flexor (AF), main flexor (F) and extensor (E) muscles in the meropodite of intact crustaceans. (*a*) Extracellular (a.c.) e.m.g.s in the crayfish, *Astacus leptodactylus*, recorded with bipolar electrodes inserted through holes drilled in the exoskeleton of the cheliped. (*b*) Intracellular (d.c.) recordings from an exposed AF muscle fibre in a posterior leg of the shore crab, *Carcinus maenas*, using a 3 M-KCl-filled glass micro-pipette; trace 1 shows spontaneous activity in the absence of a CB length change, and trace 2 (10 sec later) the response to the trapezoid length change shown below. Note the facilitating e.j.p.s in both (*a*) and (*b*), and summation of closely spaced e.j.p.s in (*b*). Length changes applied to the severed distal end of CB, clamped in fine forceps mounted on a servo-controlled puller.

in close temporal relation with a single flexor motoneurone (cf. Angaut-Petit *et al.* 1974). Any ongoing activity in these two efferents was generally inhibited by stretching CB (Fig. 1). CB release evoked strong reflex discharge of the AF excitor, at a frequency directly related to velocity of release. One (of four) excitatory motoneurone of the main flexor also responded to CB release, though at a lower frequency than the AF efferent. In contrast, one (of two) extensor motoneurone responded only to CB stretch, again at a frequency dependent upon velocity. The AF and flexor responses were enhanced, and the extensor diminished, by increased illumination of the eyes, indicating hetero-modality modulation of proprioceptive reflexes.

The results highlight the complexity of the efferent control of individual muscle receptors, and suggest that reflex interactions between proprioceptors may be important in intersegmental coordination in jointed limbs.

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## Proliferation of mononuclear cells in skeletal muscle after denervation or muscle injury

BY ROSEMARY JONES and J. T. LANE. *Departments of Physiology and Experimental Pathology, The Medical School, Birmingham B15 2TJ*

Skeletal muscle becomes hypersensitive to acetylcholine (ACh) following denervation or muscle damage. In rat hind-limb muscles the time course of the development of the increase in sensitivity is similar regardless of the nature of the 'insult' to the muscle (Jones & Vrbová, 1973). This indicates that the development of hypersensitivity is related to a factor common to both denervation and muscle injury. Cellular infiltration in damaged muscles is known to occur (Reznik, 1969) and dividing nuclei have been observed in denervated muscles. When this mitotic activity is prevented the development of denervation hypersensitivity is inhibited (Blunt & Jones, 1972). The accumulation of cells within muscle, and the development of hypersensitivity appear, therefore, to be related.

It is possible to separate alien cells from muscle tissue, and we have taken advantage of this to examine the cell population in normal rat muscles, and muscles which are hypersensitive to ACh.

Adult rats were operated under ether anaesthesia. In one group the sciatic nerve was sectioned in the thigh. In a second group a small piece of nerve was excised from the brachial plexus and placed on to the

surface of the soleus muscle in one leg. Three days later, when the sensitivity of operated muscles from both groups approached maximum (Jones & Vrbová, 1973), soleus muscles were removed from both legs. In separate containers, control and operated muscles were teased apart and gently agitated in HEPES buffered RPMI medium for 30 min at 37° C (see Lane & Ling, 1973). The medium was separated from the muscle tissue and centrifuged to precipitate a cell pellet. The muscles were then incubated for a second time in medium containing 0·25% trypsin.

Cell counts performed on resuspended cell pellets showed that in both groups of operated muscles the number of cells present was nearly 10 times greater than in control muscles. Of the total number of cells extracted the majority were obtained after the first incubation period, without trypsinization, indicating that the cells were easily removed from their sites within the muscle. Cell types found were monocytes, some lymphocytes and polymorphonuclear leucocytes.

Thus an increase in the number of mononuclear cells present in skeletal muscle is seen at a time when the muscles have become hypersensitive to ACh. The cells may increase in number as a result of local cell division, or may be blood-borne. In rats, denervation hypersensitivity fails to develop when cell division is arrested by cytotoxic drugs. Recent experiments show that denervated muscles removed from rats treated with doses of cytotoxic drugs previously found to inhibit denervation hypersensitivity do not yield more cells than innervated muscles from the same animals.

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## A model for the procaine end-plate current

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The decay of the end-plate current (e.p.c.) recorded in voltage-clamped frog muscle is normally a single exponential corresponding to the channel closing rate (Magleby & Stevens, 1972). However, in the presence of procaine the e.p.c. decay shows two components – an early component which is faster than the control decay, and a later component which is slower

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(Kordas, 1970). The following model of the procaine e.p.c. is based partly on Armstrong's (1971) theory of alkyl triethylammonium action on axonal delayed rectifier channels, and partly on an analysis of similar effects produced by barbiturates at frog end-plate (Adams, 1974).

It is proposed that part of the procaine molecule (presumably the cationic head) can transiently bind to and block open but not closed end-plate channels. The early component of the procaine e.p.c. tail therefore represents channels either being blocked, or closing in the normal manner. The later component arises because as the open but blocked channels progressively unblock (procaine dissociating) they are left in the open state, and only close again at the normal rate. Also, they may again be blocked by procaine before closing has occurred.

Since the postulated binding is within the channel and is partly coulombic, the binding rate constants will be voltage-dependent (Woodhull, 1973). The model therefore predicts that the procaine e.p.c. tail will be affected by membrane potential, in a manner that agrees with experiment. For example, hyperpolarization tends to increase the rate of association, which opposes the normal tendency of the channels to remain open longer (Magleby & Stevens, 1972), so that the early decay may even be accelerated by hyperpolarization. Because hyperpolarization slows the dissociation of procaine the slow component of the e.p.c. will be prolonged. However, by the same token at any moment less channels are left open by dissociating procaine, and so the amplitude of the slow component may even decrease, despite the increased driving electrochemical gradient. The peak conductance of the procaine e.p.c. decreases with hyperpolarization because the increased rate of block means that more channels have blocked by the time the peak is reached. Depolarization speeds the slow component and slows the fast component, so that at potentials near the sodium equilibrium potential the tail decay approaches a single exponential. All these phenomena are observed experimentally (Kordas, 1970; Maeno, Edwards & Hashimura, 1971; Deguchi & Narahashi, 1971). The model leads to a set of equations that permit the forward and reverse rate constants for procaine binding to be estimated at about $10^7$ $M^{-1}$ $s^{-1}$ and 300 $s^{-1}$ respectively.

Lignocaine probably acts similarly but dissociates rather more slowly. Thus at the resting potential the slow component of the lignocaine e.p.c. is very small, and at positive membrane potentials the amplitude of the slow component increases sufficiently to produce an e.p.c. that resembles the procaine e.p.c. recorded at the resting potential.

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## Evidence of a non-linear voltage dependence of the additional current flowing in the membrane of smooth muscle when the acetylcholine receptor is stimulated

BY T. B. BOLTON. *University Department of Pharmacology, Oxford*

In a recent communication (Bolton, 1974*a*) it was described how voltage-clamp experiments on smooth-muscle strips from longitudinal muscle of ileum, using a double sucrose-gap method with extracellular recording (Rougier, Vassort & Stämpfli, 1968), did not reveal any appreciable voltage dependence of the additional conductance appearing in the membrane in the presence of carbachol. However, the narrow strips used in the experiments, combined with a small node width (0·2 mm), had the effect that the application of carbachol did not produce the typical oscillations which are characteristic of its action on larger pieces of ileal smooth muscle (Bolton, 1971), probably because of the large leakage current which is present in nodes which are small compared to the space constant of the muscle (McGuigan, 1974). For this reason experiments were done using a voltage-clamp technique recently described to The Society (Bolton, 1975) in which the membrane potential within the node is recorded by micro-electrode. This technique has the advantage that, by injecting current into the node across both sucrose gaps, node width may be increased to 0·5 mm, and also, by exploring the node with a second micro-electrode, it is possible to check the uniformity of potential across the node during voltage clamp.

Using this technique, experiments were done to study the action of carbachol ($1–5 \times 10^{-6}$ g/ml.) on the current–voltage relationships of longitudinal smooth muscle from taenia and ileum in hypertonic solution. Ramp command potentials were applied under voltage clamp. It was shown in several experiments by recording the potential at another point within the node by a second, independent, micro-electrode that, at the rate of ramp-rise used (about 25 mV/sec), the potential at any instant showed insignificant variation across the node. Two ramps, one depolarizing, one hyperpolarizing, were sufficient to obtain the current–voltage relationship over a wide range. The current–voltage relationship was

obtained before, in the presence of, and sometimes following, the application of carbachol while recording from the same cell. When leakage current across the sucrose gaps was small, the additional current flowing upon the application of carbachol was not a simple linear function of potential as observed in previous experiments using small nodes (Bolton, 1974) except in a hyperpolarizing direction. In the range between zero and the resting membrane potential, the additional inward current appearing in the presence of carbachol increased as the membrane potential was decreased towards zero. This behaviour is of a type that might explain the increase in the slow potential and the oscillation of the membrane potential which carbachol (or acetylcholine) produces in smooth muscle (Bolton, 1971).

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## Effects of acetylcholine on membrane currents in frog atrial muscle

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Acetylcholine (ACh) produces three major electrophysiological effects in atrial muscle: (1) a small hyperpolarization of the resting potential, (2) a decrease in action potential height and duration, and (3) a reduction in twitch amplitude. These changes have previously been attributed to an increase in membrane potassium permeability (see Hutter, 1957; Trautwein & Dudel, 1958).

The present experiments studied the effect of ACh on ionic currents which generate the atrial action potential. Membrane currents were recorded from atrial trabeculae of the bullfrog, *Rana catesbeiana*, using a double sucrose gap voltage-clamp technique (Brown & Noble, 1969). The width of the test region was about 200 $\mu$m and the trabeculae ranged in thickness from 100–250 $\mu$m.

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Exposure of the test region to ACh ($8 \times 10^{-7}$ M) increased the apparent membrane conductance near the resting potential twofold. This increase is consistent with the elevation of $^{42}K$ loss by ACh in tortoise auricle (Harris & Hutter, 1956). In the present case, the real effect may be larger, since changes in genuine membrane current were probably masked by shunt current in the sucrose regions.

The experiments also revealed an unexpected result: ACh markedly reduced the slow inward current (Rougier, Vassort, Garnier, Gargouil & Coraboeuf, 1969). The first hint of this appeared when studying activity

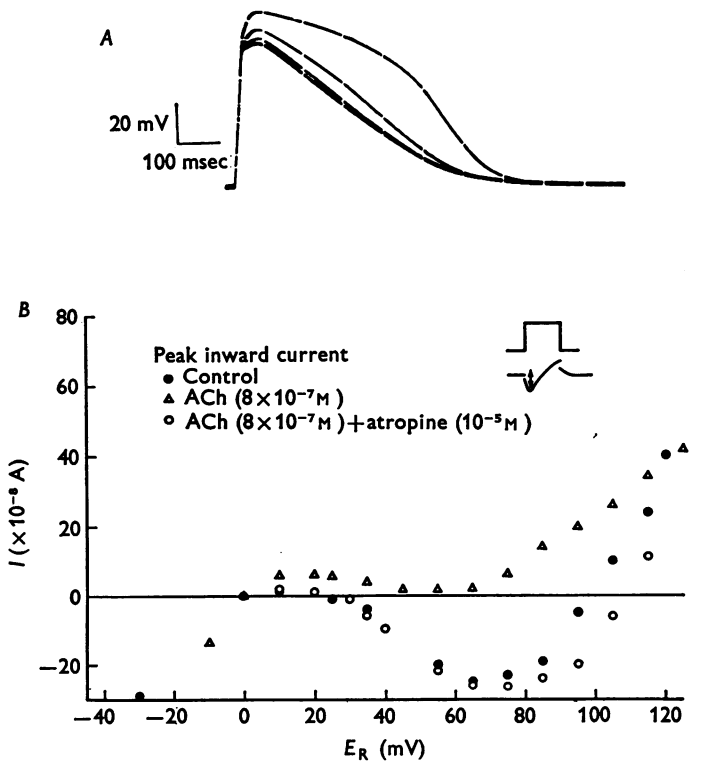


Fig. 1. (*A*) Atrial action potentials in Ringer solution containing $3 \times 10^{-6}$ M tetrodotoxin (TTX). The highest action potential was recorded before exposure to ACh ($8 \times 10^{-7}$ M). Progressively lower and briefer action potentials were recorded 2, 4 and 8 min after ACh application. (*B*) Effect of ACh on early ionic current (slow inward current) in Ringer with $3 \times 10^{-6}$ M TTX. Currents were recorded during step voltage changes from the holding potential (= resting potential = −75 mV). The peak inward or minimum outward current was measured as indicated by the inset, and plotted (ordinate) as a function of the depolarization (abscissa). The current–voltage relations have *not* been corrected for leakage currents, it is therefore likely that the magnitude of the inward currents have been underestimated.

in the presence of tetrodotoxin. Since tetrodotoxin abolished the fast sodium current, the resulting action potentials were dependent on the slow inward current, and, as illustrated in Fig. 1*A*, their height was very sensitive to ACh. More direct evidence was provided by voltage-clamp experiments in tetrodotoxin. Fig. 1*B* shows that ACh decreased the inward current peak which follows step depolarizations from the resting potential. The voltage dependence of the ACh effect resembles that of the slow inward component, indicating that this component is ACh-sensitive. A large increase in $g_K$ cannot be the sole explanation since ACh produced little change in the early current at strongly depolarized potentials ( +120 mV) where the potassium driving force is very large.

Since the slow inward current is partly carried by $Ca^{2+}$ (Rougier *et al.* 1969), these results provide a new and rather direct explanation for the decrease in plateau height and negative inotropic effect of ACh at this concentration.

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## Effects of lignocaine (lidocaine) on the endocardial Purkinje fibres of normal and infarcted hearts

BY J. D. ALLEN, F. J. BRENNAN and A. L. WIT. *Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, and the Department of Physiology, The Queen's University of Belfast*

Portions of endocardium (5 × 4 cm) were removed from the left ventricle of normal dogs, and of dogs 1 or 3 days after ligation of the anterior descending branch of the left coronary artery. The tissue was perfused *in vitro* with Tyrode solution (K 4·0 mM/l.) equilibrated with a 5% carbon dioxide and 95% oxygen mixture. Intracellular action potentials were recorded with glass micro-electrodes filled with 3 M-KCl solution.

In normal hearts lignocaine hydrochloride (4·68 mg/l.) shortened the action potential duration of cells on the tip of the anterior papillary muscle, and did not decrease the maximum rate of depolarization. Similarly in the non-infarcted areas of infarcted hearts, lignocaine (2·5–10·0 mg/l.) shortened the action potential duration at 50% and 90% repolarization, but did not significantly reduce the maximum rate of depolarization of cells on the tip of the anterior papillary muscle or high

on the ventricular septum. Towards the apex of the normal hearts, in the area infarcted in the other preparations after ligation of the coronary artery, lignocaine (4·68 mg/l.) decreased the duration of the action potential to 50 % but not to 100 % repolarization, and did not significantly alter the maximum rate of depolarization.

Friedman, Stewart, Fenoglio & Wit (1973) have shown that a layer of damaged but viable Purkinje fibres, 1–2 cells thick, survives over the area of infarction. Deep ventricular muscle fibres are electrically and histologically dead. In infarct areas with action potential amplitude more than 100 mV, lignocaine (2·5–10·0 mg/l.) reduced the maximum rate of depolarization and slightly shortened the pathologically long action potentials. In depressed areas, with action potential amplitude less than 100 mV, the low resting membrane potential was not increased, and the overshoot and maximum rate of depolarization further decreased after lignocaine (2·5–10·0 mg/l.).

The effects of perfusion with lignocaine (5·0 mg/l.) were related to the initial mean resting membrane potential of the infarct cells. The reduction in overshoot and the percentage reduction in maximum rate of depolarization varied inversely, and the reduction in action potential duration at 50 % and 90 % repolarization varied directly with the initial mean resting membrane potential.

Abnormal pace-maker activity in the infarcted areas was not suppressed by lignocaine (4·68 mg/l.). Although pace-maker activity generally continued despite perfusion with 10·0 mg/l., it was possible on some occasions to stop it by a period of rapid stimulation. A low-amplitude oscillatory after-potential was followed by electrical quiescence; further electrical stimulation produced a return of independent pace-maker activity.

These results show that the plasma concentrations of lignocaine used in clinical practice (1·2–5·0 mg/l; Koch-Weser, 1972) do not increase the resting membrane potential of depressed cells and do not abolish abnormal automaticity in infarcted tissue. This may be one cause of persistent lignocaine-resistant ventricular arrhythmias in patients with acute myocardial infarction.

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## Rapid 'give' of sarcomeres and tension changes during servo-controlled stretches applied to contracting frog's muscle

By F. W. Flitney and D. G. Hirst. *Department of Physiology, University of St Andrews, Fife, Scotland*

In a previous communication to the Society (Flitney & Hirst, 1974) we showed that a contracting frog muscle exhibits short-range elastic properties. The stiffness of the component responsible for this behaviour decreased with increasing sarcomere length, and this led us to conclude that the elasticity in question is located in the myosin cross-bridges. In recent experiments, longer stretches (up to 1 mm) were applied at the peak of a tetanus, and simultaneous recordings of tension and sarcomere extension were made. The tension response shows two discontinuities: $S_1$, which represents the elastic limit of the short-range component, and $S_2$ (Fig. 1*C*, 2). Cine-photographic recordings of laser diffraction spectra during stretch show that there is an abrupt increase in the length of the sarcomeres (rapid 'give') which coincides with the point $S_2$ (Fig. 1*A*, $\Delta s$). The sarcomere extension–muscle tension curve for the results of Fig. 1*A* is shown in 1*B*. The elastic modulus of the sarcomeres ($\Delta P/\Delta l$) during the early part of the stretch is $1{\cdot}23 \times 10^{13}$ N m$^{-2}$ per m extension, and it is calculated from this that the stiffness of a single cross-bridge is about $2{\cdot}84 \times 10^{-4}$ N m$^{-1}$, a figure which compares closely with A. F. Huxley & Simmons's (1972) estimate of $2{\cdot}5 \times 10^{-4}$ N m$^{-1}$.

Our results are consistent with Huxley & Simmons's model for the mechanism of force production by cross-bridges. We propose that the short-range stiffness is due to extension of their AB linkage *without* rotation of the attached myosin head (see their fig. 5), but that further extension forces the head to rotate in the reverse direction. Length changes in excess of 7·5–8·5 nm per half-sarcomere result in cross-bridge detachment, and the potential energy stored in the extended series elastic elements is dissipated during rapid 'give' of the sarcomeres.

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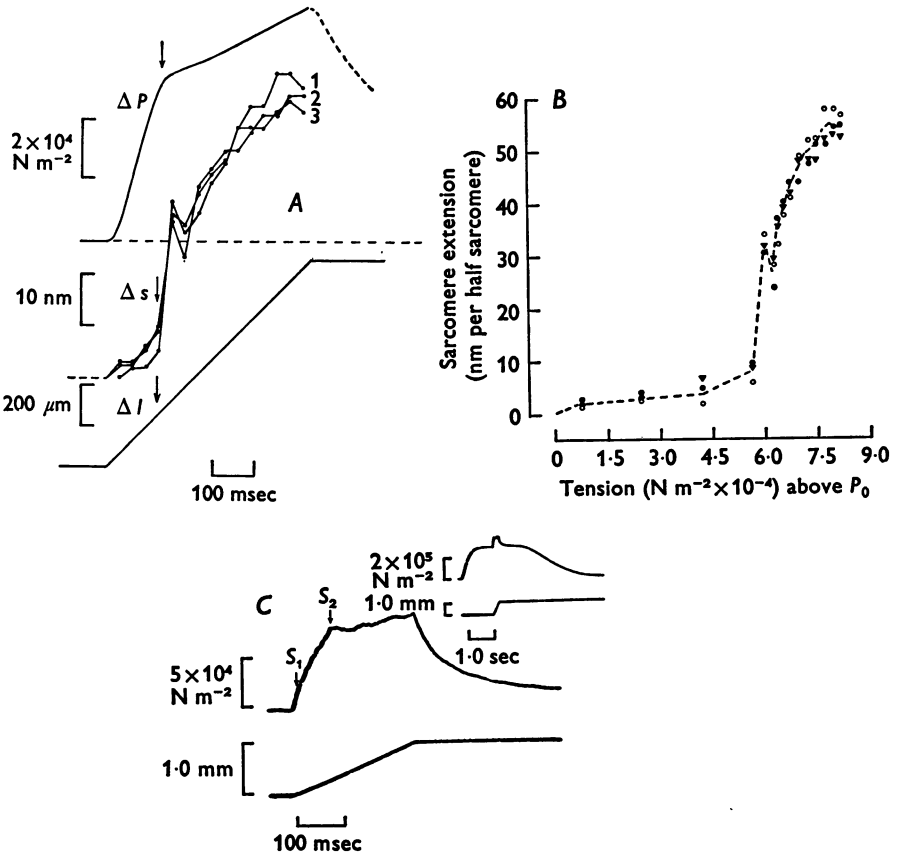


Fig. 1. (*A*) Tension ($\Delta P$) and sarcomere extension ($\Delta s$) during a ramp and hold stretch ($\Delta l$) of 1076 $\mu$m (velocity: 4304 $\mu$m $s^{-1}$) applied to a tetanized sartorius muscle (stimulation: 100 Hz, 12 V/cm). Temp., 21° C. Vertical arrow marks point at which sarcomeres show rapid 'give'. Sarcomeres sampled at three levels in the muscle: (1) 8 mm from pelvic bone; (2) 16 mm; (3) 24 mm. Muscle length, 25 mm. Initial sarcomere length, 2·59 $\mu$m. Internal shortening during rising phase of tetanus equivalent to 4·3 % of muscle length.

(*B*) Sarcomere extension–muscle tension curve showing increase in compliance of sarcomeres which occurs when the filaments are displaced by 7·5–8·5 nm per half sarcomere. Dashed line is mean of three runs.

(*C*) Typical tension record illustrating the two points of inflexion, $S_1$ and $S_2$; the whole tetanus is also shown (inset).

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## Effect of procaine and tetraethylammonium on $^{42}K$ efflux from smooth muscle of sheep carotid arteries

By W. D. Cooper, P. J. Goodford, C. C. Hardy, Jeanne Herring, C. R. K. Hind and W. R. Keatinge. *Department of Physiology, London Hospital Medical College, Turner Street, London E1 2AD*

Although the action potential of smooth muscle of sheep carotid arteries is largely Na-based, it resembles the Ca-based action potential of crustacean muscle (Fatt & Katz, 1953; Hagiwara & Nakajima, 1966) in not being blocked by moderate concentrations of procaine (Keatinge, 1968*a*, *b*, 1974). Procaine and tetraethylammonium in fact facilitated electrical activity in the arteries and the present experiments were designed to test

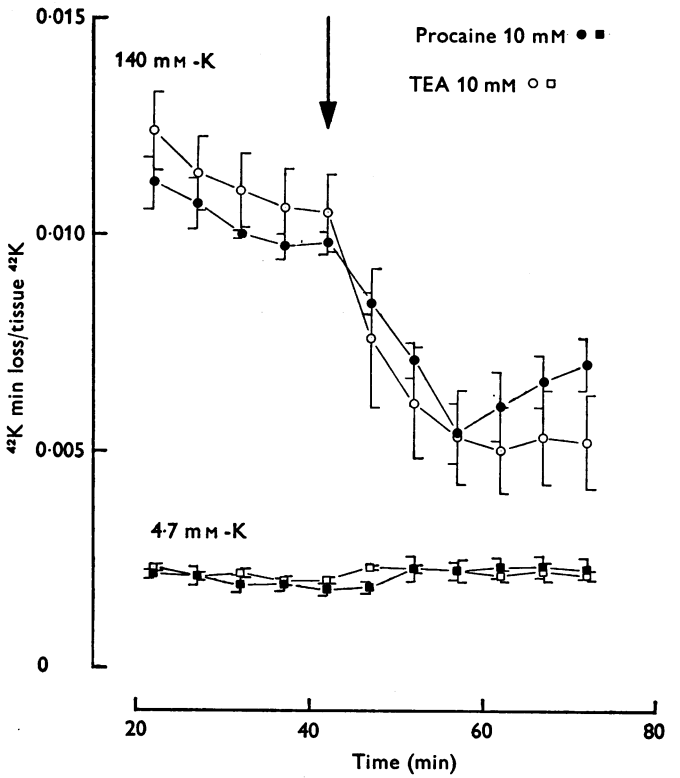


Fig. 1. Effect of procaine and of tetraethylammonium (TEA) on $^{42}K$ loss from artery strips into saline solution (NaCl, 133; $NaHCO_3$, 15; KCl, 4·7; $CaCl_2$, 1·25; glucose 7·5 mM) and into K-rich solution (NaCl, 4·7; $KHCO_3$, 15; KCl, 133; $CaCl_2$ 1·25; glucose, 7·8 mM). $^{42}K$ loss is loss$^1$.min$^{-1}$.tissue $^{42}K^{-1}$. Time is minutes after transfer from loading solution (standard saline with $^{42}K$).

whether they did so, as they do in crustacean muscle, by blocking K channels.

Helical strips of sheep carotid artery approximately 20 mm long and 1·5 mm wide were mounted under tension of 2·5 g, loaded with $^{42}K$ for 3 hr, and then transferred at 5 min intervals along a series of tubes each containing 8 ml. of inactive standard saline (K 4·7 mM) or K-rich solution (K 140·0 mM). $^{42}K$ in each tube and $^{42}K$ remaining in the artery at the end of the experiment were then measured. Fig. 1 shows that after 20–40 min the rate of loss of $^{42}K$ was much higher into K-rich solution than into standard saline. Addition of procaine or tetraethylammonium 10 mM greatly reduced $^{42}K$ loss into K-rich solution but did not reduce loss into standard saline. They generally increased the latter slightly. Procaine 1·0 mM had little effect on $^{42}K$ loss into K-rich solution, although tetraethylammonium 1·0 mM reduced it.

The decreased rate of $^{42}K$ loss produced by procaine or tetraethylammonium in K-rich solution and the absence of such a decrease in standard saline are compatible with the drugs inhibiting depolarization-induced K permeability in the arteries. The small increase in $^{42}K$ loss that procaine and tetraethylammonium sometimes induced in arteries in standard saline may be secondary to the electrical activity that they sometimes induce in this solution (Keatinge, 1974).

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## A mechanism by which mechanical disturbances can increase the uptake of macromolecules by the artery wall

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Artery wall uptake of certain macromolecules appears to be primarily passive (Siflinger, Parker & Caro, 1975). Damage to or removal of arterial endothelial cells greatly increases macromolecule uptake (Fry, 1973), implying that these cells provide the main transport resistance. Uptake is also increased by mechanical disturbances which do not appear to

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damage the endothelial cells, including appropriate levels of wall shear stress (Fry, 1973; Caro, 1973) and oscillations of pressure and flow (Nerem, Polsley, Robinson & Carey, 1974), though the mechanism is unknown. Ultrastructural studies (Stein & Stein, 1973) indicate that larger macromolecules enter arteries principally via endothelial pinocytic vesicles, rather than via inter-cell clefts.

Based on the observation that endothelial cells can deform with mechanical deformation of an artery and the proposal that pinocytic vesicles have Brownian motion (Casley-Smith, 1963) the third author has developed a preliminary continuum hydrodynamic interaction theory to describe the constrained Brownian motion of pinocytic vesicles between two closely spaced boundaries, with the operation of van der Waals attractive forces. Preliminary experiments have been conducted to test effects of mechanical disturbances on [$^{125}$I]albumin uptake by isolated dog common carotid arteries.

The main results of the theory are that (i) the flux of endothelial cell pinocytic vesicles is doubled approximately if the average trans-cell vesicle diffusion time ($\tau_d$) is effectively reduced to zero, say by mechanically induced intracellular mixing, without change of the average time of vesicle attachment to the luminal and abluminal membranes ($\tau_a$), thus effectively doubling endothelial transport of macromolecules, (ii) this increase occurs over a one order of magnitude change of frequency for simple harmonic (shm) disturbance of appropriate fixed amplitude and (iii) the change of uptake should not occur for pulsed (abrupt) disturbances of the same amplitude and frequency, because of rapid viscous decay of intracellular motions.

The artery segments were mounted at *in vivo* length ($l_0$) in a rig, filled with serum containing the label, and incubated at 37° C in saline (transmural pressure $\approx 1$ cm $H_2O$). 29 test segments (12 dogs) were subjected to shm shortening with $\frac{1}{2}\Delta l/l_0 \approx 0{\cdot}04$, and vibration period ($T$) being varied in different experiments from 0·3 to 300 sec. The ratio average permeability of test segments to average permeability of similar number of control segments for the same dog was 1·61 for $T < 20$ sec and 1·06 for $T > 20$ sec, with the means being significantly different ($P < 0{\cdot}02$). Steady stretch of this magnitude negligibly alters artery permeability to albumin (Fry, 1973). 16 other segments (3 dogs) were studied with a small tracer molecule, [$^{14}$C]sodium acetate, expected to enter the wall mainly via the inter-cell clefts, rather than via pinocytic vesicles. The permeability ratio was 1·04 for $T < 20$ sec, which is significantly less than for the comparable [$^{125}$I]albumin studies ($P < 0{\cdot}005$); the dimensions of endothelial inter-cell clefts are apparently unaffected by distension of an artery. In pulsed experiments (2 dogs) $T$ was 2 sec, but the pulse, of similar amplitude

to that above, did not exceed 0·2 sec duration, and permeability to [$^{125}$I] albumin was not different from that of controls.

The experimental findings are in general consistent with the theoretical predictions, but there is a need to extend understanding of the mechanics and to assess physiological relevance, in the light of the naturally occurring large pressure and flow oscillations.

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## Membrane properties during myotome formation in tadpoles of *Xenopus laevis* and *Bombina bombina*

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The axial musculature of the tadpole develops from paraxial mesoderm, which lies on either side of the notochord. The medial, inferior cells form myotomes and the outer, superior cells the dermatome. Segregation into somites being at late neural fold stages. In *Bombina* presumptive myotome and dermatome segment together to form somites, whereas in *Xenopus* dermatome cells remain as a continuous layer above the segmenting myotomes (Hamilton 1969). About 150 presumptive myotome cells elongate and rotate in a block so that the long axis of each cell lies parallel to the notochord; each somite is one cell long.

Jelly and vitelline membranes were stripped from embryos in Ringer solution after the neural tube had closed. Ectoderm overlying the dermatome was removed; sometimes dermatome cells were also removed to reveal the myotomes. Segmented somites lie at the cranial end while segmentation and cell rotation continues caudally. Electrical properties were determined using intracellular, high resistance, low tip potential, glass micro-pipettes.

Dermatome cells of *Xenopus* (diameter $\sim$ 10 $\mu$m) had membrane

potentials between $-6$ and $-48$ mV, mean $-25 \pm 0{\cdot}6$ mV (S.E.M., $n = 325$). Myotome cells had internal potentials between $-24$ and $-94$ mV, mean $-65 \pm 1$ mV (S.E.M., $n = 246$). Cells in segmented and unsegmented myotomes had similar membrane potentials. Current injected into a dermatome cell spread at least 350 $\mu$m within the dermatome, but did not spread down into myotome cells either before or after formation of myotomes. Neural tube, notochord and endoderm cells were also electrically insulated from each other and from dermatome and myotomes. Similar results were obtained in *Bombina*. This finding contrasts with the chick embryo where low electrical resistance connexions between these structures persist for some time after morphological differentiation has begun (Sheridan, 1968).

In both *Xenopus* and *Bombina* presumptive myotome cells were electrically coupled to each other before somite formation. After myotome alignment low resistance pathways were again found between myotome cells, both between cells in the same somite and from one somite to the next; electrotonic potentials could be recorded several somites away from the current-injecting electrode. Coupling between somites was still present just before hatching, when some motor innervation is already present (Lewis & Hughes, 1957). During segmentation and alignment of myotome cells a complex sequence of changes in electrical coupling occur in which segmenting cells uncouple from unsegmented mesoderm and subsequently form new contacts with the already aligned myotomes.

*Xenopus* tadpoles make primitive swimming movement before extensive myotome innervation and spontaneous action potentials could then be recorded. These were blocked by 1 mM-$Mn^{2+}$, but not by tetrodotoxin. Low resistance intercellular pathways would allow transmission of excitation from one myotome to the next via a non-neural pathway.

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We are indebted to Dr J. Cooke of the National Institute for Medical Research, who kindly provided *Bombina bombina* embryos.

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## Effect of salmon calcitonin on calcium and sodium efflux in the European eel (*Anguilla anguilla* L.)

BY CHRISTOPHER G. DACKE. *Department of Physiology, Marischal College, University of Aberdeen*

Calcitonin (CT) has rapid hypocalcaemic and natriuretic actions in mammals. Sub-mammalian vertebrates show no consistent hypocalcaemic response (Dacke, 1972), due perhaps to their high levels of plasma CT (Kenny, Boelkins, Dacke, Fleming & Hansen, 1972). High plasma levels of CT are also found in Japanese eels (Orimo, Ohata, Fujita, Yoshikawa, Higashi, Jinnosuke, Watanabe & Otani, 1972). These levels are higher in sea water (SW) than fresh water (FW) adapted eels. Recently Fleming, Brehe & Hanson (1973) reported that CT stimulated $^{45}Ca$ uptake and altered $^{45}Ca$ distribution between calcified and soft tissue compartments in FW adapted Killifish (*Fundulus kansae*). This response was only apparent in the summer months and disappeared in winter.

TABLE 1. Counts/min.ml. were calculated for each 10 min time interval. Lines of best fit were computed for each fish for each of the two periods (pre- and post-treatment) in order to obtain average efflux rates. The slopes of the two lines were compared and expressed as a ratio and the ratios for CT- and control-treated fish compared by means of paired *t* tests

| | $^{45}Ca$ | | $^{22}Na$ | |
|---|---|---|---|---|
| | Controls | CT | Controls | CT |
| No. | 14 | 14 | 8 | 8 |
| Mean ratio: $\frac{\text{Post-treatment efflux}}{\text{Pre-treatment efflux}} \times 100\%$ | 126 | 98 | 148 | 154 |
| | $P < 0{\cdot}01$ | | NS | |

We are carrying out similar investigations of the covert responses to CT in European eels. Preliminary data concern the effect of CT on $Na^+$ and $Ca^{2+}$ total efflux rates. Eels in the silver condition (weight range 15–70 g) were obtained from local rivers and maintained in FW at least 2 weeks before use. At the beginning of the experiments the eels were loaded with either $^{22}Na$ or $^{45}Ca$ (25 $\mu$Ci/100 g body weight I.P.) and placed in individual tanks containing 2 l. FW. After a 1 hr equilibration period 0·5 ml. samples of water were removed from the tanks at 10 min intervals for 2 hr (pre-treatment period). The fish were then injected with a large dose of synthetic salmon CT (500 ng/100 g, I.P.) dissolved in 0·1 M formic acid +0·1 % serum albumen. Control fish received vehicle alone. Samples of water were removed for a further 3½ hr (post-treatment period). The samples were dried and counted in a gas flow counter (Nuclear Chicago Inc.).

The results (Table 1) indicate that total $^{45}Ca$ efflux is reduced in CT-treated eels compared with controls. $^{22}Na$ efflux is not affected. The $^{45}Ca$

response may reflect a change in calcified tissue turnover, or an action at the branchial or renal level.

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## Bile secretion in the anaesthetized Adelie penguin (*Pygoscelis adeliae*)

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Adult Adelie penguins weighing from 3·1–5·3 kg, were anaesthetized with sodium pentobarbitone (25 mg/kg), given intraperitoneally. The neck of the gall-bladder was ligated and the two bile ducts cannulated. Bile flowing from the cannulae was collected and the rate of its secretion measured with a drop counter. The entero-hepatic circulation of bile constituents was therefore interrupted.

Bile secretion slowly increased to reach a constant rate of between 0·03 and 0·06 ml./min$^{-1}$, 1 hr after cannulation. For the next 3 hr the secretory rate was constant with only minor variation. In one experiment the volumes of bile (in ml.) produced in successive 15 min periods were as follows: 0·42, 0·59, 0·74, and then over the next 3 hr an average of 0·91 (S.D. 0·06) per 15 min.

In mammals, under similar experimental conditions, the interruption of the entero-hepatic circulation leads to a progressive diminution of bile flow, due to the decreasing rate of bile salt excretion (Wheeler, 1965). In the penguin, however, it appears that there is an important secretory mechanism which is not bile salt dependent. Clearly it was of interest to test the choleretic effects of bile salts in this species. The effect of bile salts on bile production was investigated by means of certain mono-, di- and trihydroxy C 24 bile acids and their conjugates. The purified bile salts were dissolved in 0·9% saline and injected through an indwelling axillary venous cannula. Volumes between 0·25 and 3·0 ml. were injected over 10–20 sec. All the tested bile salts produced a choleresis. Few of them had an effect at doses of less than 10 mg. Each test dose was interspersed with an equal volume of 0·9% saline as a control. Sodium cholate

(trihydroxy), a normal constitutuent of bile in the penguin (Haslewood, 1967) produced an increase in secretion within 30 sec; sodium taurohyodeoxycholate (dihydroxy) had a similar effect. Sodium taurolithocholate (monohydroxy) induced virtually complete cholestasis for 20 min, followed by a period of choleresis, the bile secretion rate rising to 0·17 ml. $min^{-1}$, a 250% increase, and staying at the higher rate for about half an hour before returning to the original rate.

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## Circulating noradrenaline in the potentiation by hypoxia of the hyperventilation in exercise

BY L. J. CLANCY, J. A. J. H. CRITCHLEY and A. G. LEITCH. *Department of Medicine, Royal Infirmary of Edinburgh, Edinburgh and Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh*

The respiratory minute volume ($\dot{V}_E$) is greater in hypoxic than in normoxic exercise by an amount which increases with the intensity of the work (Asmussen & Nielsen, 1958). The mechanism of this potentiation is unclear. It is known that arterial catecholamine levels increase with exercise in man and that the rise is related to the level of work (Häggendal, Hartley & Saltin, 1970). Hypoxia has also been shown to raise plasma catecholamine levels (Cunningham, Becker & Kreuger, 1965). We wondered whether circulating catecholamines might contribute to the interaction of stimuli in hypoxic exercise.

We estimated the arterial noradrenaline concentration in four normal adult males at three levels of oxygen uptake ($\dot{V}_{O_2}$) while standing or walking on a treadmill and breathing air or 14% oxygen. Plasma noradrenaline levels, measured by the trihydroxyindole method (Vendsalü, 1960), are shown for the four subjects. Only at the greater of the two work loads were noradrenaline levels significantly higher during hypoxia than during air breathing ($P < 0·01$, paired $t$ tests). During hypoxic exercise at a $\dot{V}_{O_2}$ of 1·5 l. $min^{-1}$, the noradrenaline concentrations were double those when the $\dot{V}_{O_2}$ was 1 l. $min^{-1}$. Furthermore, only at the highest exercise rate was $\dot{V}_E$ significantly greater in hypoxia than in normoxia.

Intravenous infusion of noradrenaline stimulates ventilation in normal resting man (Cunningham, Hey, Patrick & Lloyd, 1963; Patrick, 1964). This effect is dependent on the presence of, and increases sensitivity to, hypoxia. We estimate that the rise in arterial catecholamine concentration achieved at their lower infusion rate of 5 $\mu g.min^{-1}$ was between

1 and 1·5 ng.ml$^{-1}$. This is twice that seen in our experiments. With this rate of infusion, and at the gas tensions prevailing during exercise in hypoxia in our experiments, Patrick (1964) found that ventilation was increased by about 50%.

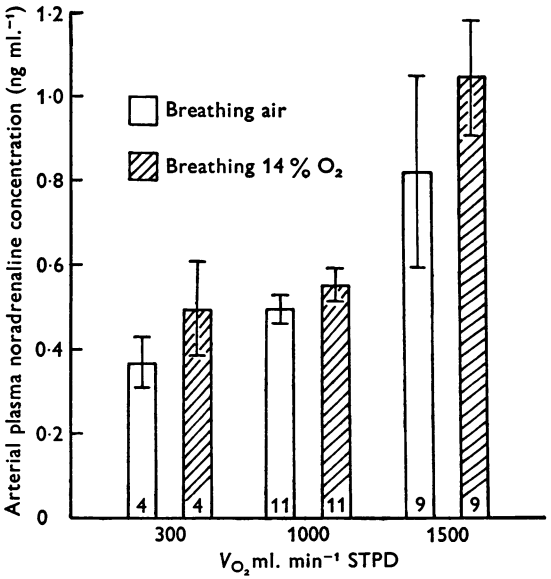


Fig. 1. Mean arterial plasma noradrenaline concentrations of four subjects at rest and at two work loads while breathing air or 14% oxygen in nitrogen.

We suggest that an elevated circulating noradrenaline concentration may have contributed to the 25% increase in ventilation during hypoxia which was seen in our subjects at a $\dot{V}_{O_2}$ of 1·5 l.min$^{-1}$.

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## Evidence that prostaglandin release mediates pulmonary vasoconstriction induced by *E. coli* endotoxin

By J. R. Parratt and R. M. Sturgess. *Department of Physiology and Pharmacology, Royal College, University of Strathclyde, Glasgow, G1 1XW*

The intravenous administration of *E. coli* endotoxin to cats results in acute pulmonary vasoconstriction and in a reduction in pulmonary compliance within 1–3 min of the injection; this usually leads to pulmonary oedema and to right ventricular failure (Parratt, 1973). Pulmonary hypertension is also a characteristic of endotoxin shock in other species, including primates. The factors responsible for this increase in pulmonary vascular resistance are uncertain. Endotoxin has no direct action on pulmonary vascular smooth muscle *in vitro* and mechanical obstruction of the smaller pulmonary vessels by platelet clumping seems unlikely since the pulmonary hypertension is unaffected by carbochromen. This drug is a potent inhibitor of platelet aggregation (Hampton, Harrison, Honour & Mitchell, 1967). A number of vasoactive substances are released by endotoxin including histamine (Vick, 1960), angiotensin, adrenaline and noradrenaline (Hall & Hodge, 1971), 5-hydroxytryptamine (Hall, Hodge, Irvine, Katic & Middleton, 1972) and prostaglandins (Collier, Herman & Vane, 1973). The following experiments provide evidence that it is the massive release of a prostaglandin that is responsible for the increase in pulmonary vascular resistance during the acute phase of endotoxin shock.

Cats were anaesthetized with sodium pentobarbitone, artificially ventilated and given heparin (500 units/kg, intravenously). Systemic arterial and pulmonary blood pressures, and cardiac output, were measured as previously described (Parratt, 1973). *E. coli* endotoxin (Difco Laboratories) injected intravenously in a dose of 2 mg/kg (ten experiments) elevated pulmonary artery pressure from $19 \pm 1$ mmHg (systolic) and $9 \pm 1$ mmHg (diastolic) to $37 \pm 4$ mmHg and $23 \pm 3$ mmHg respectively. That histamine is not the main humoral mediator involved is demonstrated by the fact that this rise in pulmonary blood pressure was unaffected either by the prolonged chronic administration of compound 48/80 (in doses sufficient to reduce skin mast cell histamine to less than 10% of control), or by the acute intravenous administration of a combination of mepyramine (2 mg/kg) and burimamide (2 mg/kg). The evidence that a prostaglandin is involved is:

(1) Pre-treatment of cats with known inhibitors of prostaglandin biosynthesis (such as indomethacin, 10 mg/kg or sodium meclofenamate, 0·5, 2·0 or 5·0 mg/kg, administered intravenously) prevented, or markedly reduced, endotoxin-induced pulmonary vasoconstriction. In the doses

used these drugs did not modify the pulmonary vascular responses to histamine, 5-hydroxytryptamine or prostaglandin $F_{2\alpha}$.

(2) Pre-treatment with prostaglandin antagonist polyphloretin phosphate (200 mg/kg, intravenously) prevented, or markedly reduced, the rise in pulmonary artery pressure induced by the intravenous administration of PGF (1·25–5·0 μg/kg) or *E. coli* endotoxin (2 mg/kg). This dose of polyphloretin did not modify the pulmonary vasoconstrictor effects of histamine or 5-hydroxytryptamine.

This work was supported by the Medical Research Council. We are also grateful to Parke, Davis and Co. for the sodium meclofenamate, to Professor B. Högberg of Leo Research for the polyphloretin phosphate and to Dr A. F. Green of the Wellcome Research Laboratories for the compound 48/80. R.M.S. is the holder of a University of Strathclyde open scholarship.

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## A preliminary investigation of prostaglandin synthetase activity in normal, sensitized and challenged sensitized guinea-pig lung

BY R. BENZIE, J. R. BOOT and W. DAWSON. *Lilly Research Centre Ltd, Erl Wood Manor, Windlesham, Surrey*

Prostaglandins are released on immunological challenge of both human and guinea-pig lung (Piper & Vane, 1971; Piper & Walker, 1973). These potent pharmacological agents are capable of inducing bronchoconstriction ($PGF_{2\alpha}$) or bronchodilatation ($PGE_2$) and the balance between these substances will presumably determine the end physiological response due to PG release. Prostaglandins are formed by an enzyme complex from unsaturated fatty acids, particularly arachidonic acid. The products of this reaction in lung have not been characterized in detail, nor have the factors controlling the ability of the enzymes to synthesize either $PGF_{2\alpha}$ or $PGE_2$. Initial studies to compare the enzyme activity in normal and sensitized guinea-pig lungs revealed changes which could relate to the pathogenesis of asthma in man.

Guinea-pigs were sensitized to ovalbumin (100 mg intraperitoneally and subcutaneously), allowed to rest for 3 weeks, sacrificed and their lungs perfused with Tyrode solution (Brocklehurst 1960). A non-sensitized

group of animals were similarly perfused. One group of sensitized lungs ($n = 5$) were challenged during perfusion with ovalbumin (5 mg), the other two groups, one sensitized, the other non-sensitized, received a control saline injection. After a further 15 min perfusion, each group of lungs were pooled and homogenized in 0·1 M phosphate buffer at pH 8.

The homogenate was centrifuged (9000*g*, 20 min, 4°), the pellet discarded and the supernatant centrifuged again (75000*g*, 120 min, 4°). The pellet was washed, resuspended in 0·01 M phosphate buffer (pH 8) and freeze dried. This powder constituted the microsomal PG synthetase preparation for each lung group.

The enzyme assay was performed at three substrate concentrations (0·2, 0·4, and 6·6 $\mu$mole/ml.) using arachidonic acid mixed with 1-[$^{14}$C]-arachidonic acid in the presence or absence of reduced glutathione (0·65 $\mu$mole/ml.).

Three radiolabelled products were formed by each lung pool; $PGF_{2\alpha}$, $PGE_2$ and a less polar compound tentatively identified as $PGD_2$.

Sensitized non-challenged lungs formed more of each radiolabelled product than the control preparations, whilst the challenged lungs had the least PG-forming capacity. The changes in prostaglandin forming capacity were only seen at low substrate concentrations, which probably relate more closely to physiological levels. In only one instance did glutathione modify the percentage conversion of arachidonate to PGs: this was at the lowest substrate concentration in unsensitized animals, and this elevated value was still increased in the sensitized lungs. Similar results were seen in two further series of perfusions.

These data suggest that the sensitization procedure increases the PG-forming capacity of the lung tissue and that challenge returns this elevated value to control levels. It is possible that changes in cell membranes associated with binding of specific antibodies modifies the availability of endogenous substrate and so increases PG synthetase activity. It is difficult to see how the sensitization process could modify the synthetase enzyme complex *per se*.

These changes in PG synthetase activity associated with the immunological reaction could imply that PGs have a causal role to play, perhaps by modifying the response of bronchial smooth muscle to other spasmogens (Dawson & Sweatman, 1975).

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