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ADRENAL CATECHOLAMINE OUTPUT IN RESPONSE TO STIMULATION OF THE SPLANCHNIC NERVE IN BURSTS IN THE CONSCIOUS CALF

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SUMMARY

1. Different patterns of stimulation have been applied to the peripheral end of the right splanchnic nerve, below behavioural threshold, in conscious 2–5-week-old calves.

2. The effects of continuous stimulation at 4 Hz for 10 min were compared with those of stimulation at 40 Hz in 1 s bursts at 10 s intervals for the same period. Delivering the same total number of impulses in the form of bursts in this way increased the output of both adrenaline and noradrenaline and this increase was statistically significant in the case of adrenaline ($P < 0{\cdot}02$) but not noradrenaline.

3. The effects of stimulation in 1 s bursts at 10 s intervals for 2–3 min were investigated over the frequency range 10–150 Hz and compared with the effects of continuous stimulation over the range 1–15 Hz obtained previously in conscious calves of the same age (Edwards, Furness & Halle, 1980). The output of adrenaline but not noradrenaline was found to be significantly greater in response to stimulation in bursts at frequencies of up to 40 Hz than when the equivalent number of impulses were delivered at a constant rate ($P < 0{\cdot}02$).

4. It is concluded that the release of catecholamines from the adrenal gland is maximal at relatively high frequencies (40–100 Hz) when the impulses are delivered in bursts.

INTRODUCTION

Recent studies of the effect of stimulation of certain parasympathetic nerves in 'bursts' has shown that some responses are potentiated under these conditions. Release of vasoactive intestinal peptide (VIP) from parasympathetic nerve terminals in the submaxillary gland of the cat is optimal at 80 Hz, when the impulses are delivered to the chorda tympani in 1 s bursts at 10 s intervals, as is the fall in submaxillary vascular resistance, in both the presence and absence of atropine (Andersson, Bloom, Edwards & Järhult, 1981*a*, *b*). Furthermore, the flow of submaxillary saliva, which is mediated by a muscarinic action of acetylcholine, is maximal in response to stimulation of the chorda tympani at 60 Hz under the same conditions in the absence of atropine (Andersson *et al.* 1981*b*). In the same species Andersson & Järhult (1981) have succeeded in obtaining complete separation of the

two non-adrenergic, non-cholinergic colonic responses to stimulation of the pelvic parasympathetic innervation (vasodilatation and colonic contraction) by varying the pattern of stimulation. Their results show that the colonic vasculature of the atropinized cat resembles that of the submaxillary gland in that stimulation in 1 s bursts at 10 s intervals at either 20 or 40 Hz is much more effective in producing vasodilatation than the delivery of the same total number of impulses in unit time at the corresponding constant frequency. In contrast, stimulation in bursts is completely ineffective in causing contraction of the viscus, although forceful contractions are readily elicited by constant stimulation of the pelvic nerve at low frequencies, and maximal responses are obtained during stimulation at 8 Hz.

The present study was undertaken in order to discover whether the release of adrenaline or noradrenaline from the adrenal medulla might also depend upon the pattern of stimulation applied to the autonomic (but in this case sympathetic) innervation. The experiments were carried out in 2–5-week-old calves because the output of catecholamines from the gland can be quantified in this species, by means of the adrenal-clamp technique (Edwards, Hardy & Malinowska, 1974), during splanchnic nerve stimulation in the conscious animal, thereby eliminating the distortions of the response which anaesthesia produces (Edwards, Furness & Helle, 1980). The protocols were arranged in such a way that the results could be compared with those of a previous study of adrenal medullary responses to stimulation of the splanchnic nerve at constant frequencies (Edwards *et al.* 1980).

Certain of these results have been published previously in a preliminary form (Edwards, 1981).

METHODS

Animals

Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging between 17 and 36 days (27·5–37·6 kg body weight). The animals were kept in individual pens in the laboratory animal house and maintained on a diet of milk (6–8 pints per day). Food was withheld for a minimum period of 14 h prior to surgery or any experiment.

Experimental procedures

Anaesthetic and surgical procedures were identical with those described previously (Edwards *et al.* 1974, 1980). Briefly, following removal of the right kidney, a specially designed clamp was implanted to enable subsequent collection of the venous effluent blood from the right adrenal gland when required. The cross-sectional area of all parts of the outflow system was at least six times greater than that of the adrenal vein, and particular care was taken to see that the tip of the collection tube was held at the level of the gland while samples were collected; the resistance of the venous conduit is therefore considered to have been negligible. Narrow bore polythene catheters were inserted into the saphenous arteries, so that the tips lay in the abdominal aorta, and used later to monitor aortic blood pressure and for collection of blood samples. The right splanchnic nerve was cut immediately below the diaphragm and the peripheral end enclosed in a fluid electrode constructed of silver wire and silicone rubber and designed to minimize spread of stimulus (Barnes, Bower & Rink, 1979). Procaine penicillin (600,000 i.u.) and dihydrostreptomycin (0·5 g; May & Baker Ltd.) were administered routinely prior to surgery.

The experiments were carried out when the animals had fully recovered from anaesthesia. The protocols were so arranged that the results could be compared with those of a previous study of adrenal medullary responses to splanchnic nerve stimulation, in conscious calves of the same age under precisely the same conditions, except that continuous stimulation at constant frequencies was invariably employed (Edwards *et al.* 1980). In each case a standard 20–30 V square-wave stimulus

(pulse width 1 ms) was delivered to the peripheral end of the right splanchnic nerve at a pre-determined frequency for 1 s at 10 s intervals either for 2–3 min or for 10 min. Animals in which the effects of stimulation for 2–3 min were investigated were tested repeatedly at 30 min intervals at different stimulus frequencies in a random order. These tests were interspersed with tests at a standard frequency (70 Hz) in order to monitor changes attributable to fatigue. In the case of stimulation for 10 min, n values refer to different individual animals as duplicate tests were avoided. Heart rate and aortic blood pressure were monitored continuously by means of a Devices L221 pressure transducer, connected to a Devices M19 recorder. Right adrenal blood flow was estimated gravimetrically and corrected for haematocrit % before the output of catecholamines from the gland was computed. Adrenal vascular resistance was calculated by dividing the perfusion pressure (aortic blood pressure) by the right adrenal blood flow. The outputs of adrenaline and noradrenaline from the gland were expressed as ng kg body wt.$^{-1}$ min^{-1}, having been estimated from the concentration of each catecholamine in the adrenal venous effluent plasma and the adrenal plasma flow.

Analytical procedures

Arterial blood samples were collected at intervals for haematocrit estimations. Right adrenal venous blood samples were collected, at the same intervals, into heparinized tubes containing EDTA for catecholamine estimations, centrifuged at +4 °C as soon as possible and the plasma was then sequestered at −20 °C. Adrenaline and noradrenaline were estimated by a modification of von Euler & Floding's trihydroxyindole method (von Euler & Floding, 1955) as previously described (Bloom, Edwards, Hardy, Malinowska & Silver, 1975). Statistical analyses were made according to the methods of Snedecor & Cochran (1967).

RESULTS

Comparison of the effects of continuous stimulation of the splanchnic nerve with those of stimulation in bursts

Stimulation of the peripheral end of the right splanchnic nerve at 40 Hz, in 1 s bursts at 10 s intervals for 10 min produced closely similar changes in a number of cardiovascular and right adrenal responses to those recorded during the corresponding constant rate of stimulation, which would deliver precisely the same number of impulses over the same period (at 4 Hz). Thus, the rise in mean aortic blood pressure, right adrenal blood flow and haematocrit % which occurred during continuous stimulation at 4 Hz were reproduced by stimulation at 40 Hz in bursts, as was the fall in mean right adrenal vascular resistance. With the exception of the values at 2½ min, closely similar changes in mean heart rate were also recorded in these two groups of animals (Fig. 1).

In contrast, delivering the same total number of impulses in the form of bursts in this way substantially increased the output of both adrenaline and noradrenaline from the right adrenal gland (Fig. 2), and this increase was greater in both proportional and absolute terms in the case of adrenaline. The resting output of both adrenaline and noradrenaline from the denervated adrenal gland of these conscious calves was invariably less than 10 ng kg^{-1} min^{-1}. During stimulation in bursts at 40 Hz the output of adrenaline was 250 ± 46 ng kg^{-1} min^{-1} at 2½ min, 264 ± 45 at 5 min, 253 ± 44 at 7½ min and 239 ± 43 at 10 min ($n = 9$), whereas the corresponding values obtained previously during continuous stimulation at 4 Hz were 142 ± 13, 125 ± 13, 113 ± 12 and 110 ± 13 ng kg^{-1} min^{-1} ($n = 6$); (Edwards *et al.* 1980). The corresponding values for noradrenaline output under the same conditions in the present experiments were 158 ± 24, 145 ± 23, 133 ± 17 and 109 ± 15 ng kg^{-1} min^{-1} during

stimulation in bursts and 105 ± 16, 81 ± 13, 63 ± 10 and 53 ± 11 ng kg^{-1} min^{-1} during stimulation at constant frequency (Edwards *et al.* 1980; Fig. 2).

Comparison of the same responses to splanchnic nerve stimulation at higher frequencies, 100 Hz in 1 s bursts at 10 s intervals, or 10 Hz continuously, showed that the outputs of both catecholamines during stimulation in bursts were closely similar

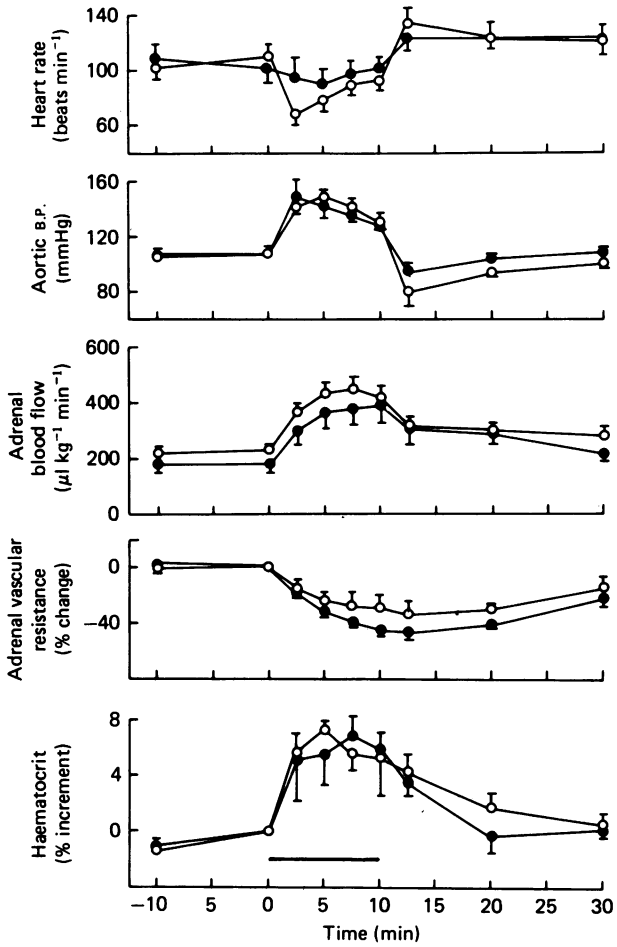

Fig. 1. Comparison of cardiovascular and right adrenal responses of conscious 2–5-week-old calves to stimulation of the peripheral end of the right splanchnic nerve at either 4 Hz continuously for 10 min (●; $n = 6$), or at 40 Hz in 1 s bursts at 10 s intervals for the same period (○, $n = 9$). Horizontal bar: duration of stimulation. Vertical bars: S.E. of each mean value.

to those during continuous stimulation. Apart from the output of noradrenaline at $2\frac{1}{2}$ min, which was significantly lower during stimulation in bursts, stimulation at this very high frequency for just 10% of the time available proved to be just as effective in releasing the two amines as when the stimuli were delivered at the much lower constant frequency of 10 Hz (Fig. 3). Apart from the fall in adrenal vascular resistance, stimulation at 100 Hz in bursts was also just as effective in eliciting the other cardiovascular and adrenal responses as continuous stimulation at 10 Hz (Fig. 4).

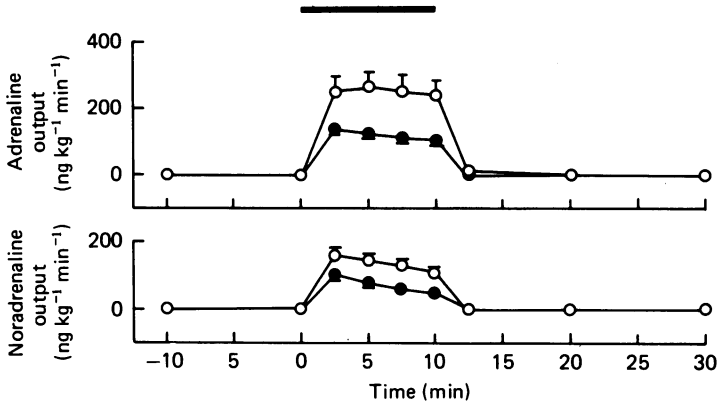

Fig. 2. Comparison of the changes in catecholamine output from the right adrenal gland of conscious 2–5-week-old calves in response to stimulation of the peripheral end of the right splanchnic nerve at either 4 Hz continuously for 10 min (●; $n = 6$) or at 40 Hz in 1 s bursts at 10 s intervals for the same period (○; $n = 9$). Horizontal bar: duration of stimulation. Vertical bars: S.E. of each mean value.

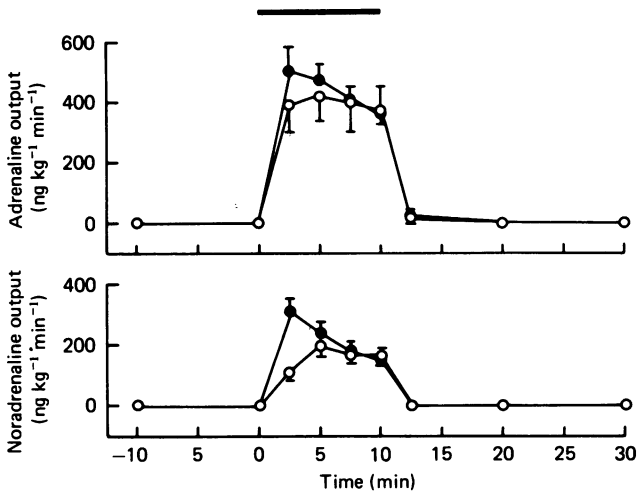

Fig. 3. Comparison of the changes in catecholamine output from the right adrenal gland of conscious 2–5-week-old calves in response to stimulation of the peripheral end of the right splanchnic nerve at either 10 Hz continuously for 10 min (●, $n = 6$), or at 100 Hz for 1 s at 10 s intervals for the same period (○; $n = 8$). Horizontal bar: duration of stimulation. Vertical bars: S.E. of each mean value.

The relation between catecholamine output and frequency of splanchnic nerve stimulation

The outputs of both adrenaline and noradrenaline from the right adrenal medulla are linearly related to the frequency at which the splanchnic nerve is stimulated when a continuous stimulus at constant frequency is employed in conscious calves (Edwards *et al.* 1980). In the present study the effects of stimulation in 1 s bursts at 10 s intervals for 2–3 min have been investigated over the frequency range 10–150 Hz and compared with the results obtained previously during continuous stimulation at

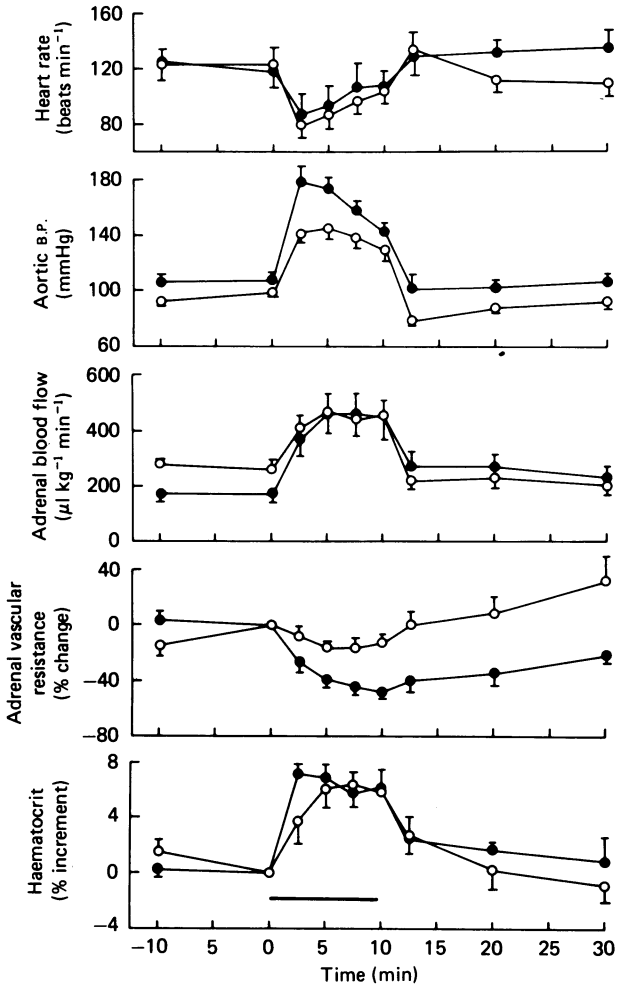

Fig. 4. Comparison of cardiovascular and right adrenal responses of conscious 2–5-week-old calves to stimulation of the peripheral end of the right splanchnic nerve at either 10 Hz continuously for 10 min (●; $n = 6$), or at 100 Hz in 1 s bursts at 10 s intervals for the same period (○; $n = 8$). Horizontal bar: duration of stimulation. Vertical bars: S.E. of each mean value.

1–15 Hz for the same period. The output of adrenaline was significantly greater in response to stimulation in bursts at 40 Hz and below than when the equivalent number of impulses were delivered at a constant rate ($P < 0{\cdot}02$). Bursts of stimuli at 70 and 100 Hz produced a similar output to that obtained in response to continuous stimulation at 7 and 10 Hz, whereas the output in response to bursts at 150 Hz was significantly less than that during continuous stimulation at 15 Hz (Fig. 5). The output of noradrenaline was not significantly increased by using bursts of stimuli at any frequency, but was significantly decreased at 70 Hz and above compared with the equivalent constant rate of stimulation (Fig. 6).

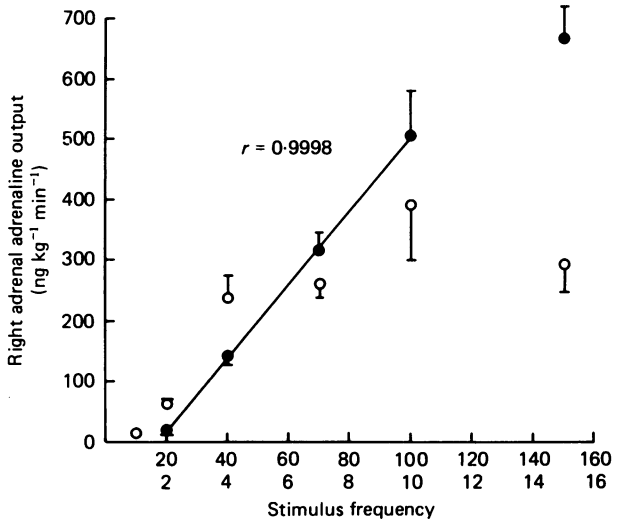

Fig. 5. Comparison of the relation between the output of adrenaline from the right adrenal gland of conscious 2–5-week-old calves, in response to stimulation of the peripheral end of the right splanchnic nerve continuously at relatively low frequencies (●; lower abscissa; from Edwards *et al.* 1980) and the corresponding higher frequency, delivered in 1 s bursts at 10 s intervals (○; upper abscissa). Vertical bars: S.E. of each mean value when in excess of the size of the symbol. Regression line calculated by the method of least squares.

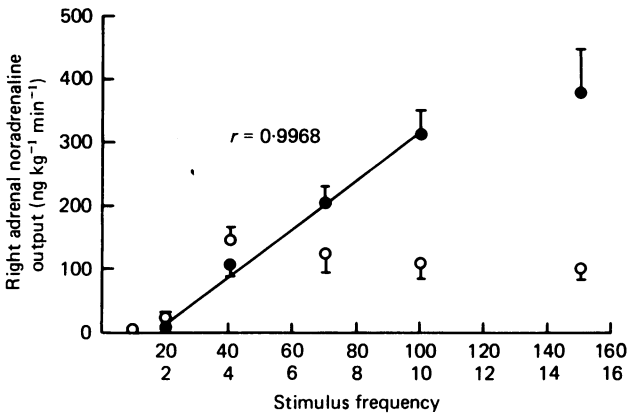

Fig. 6. Comparison of the relation between the output of noradrenaline from the right adrenal gland of conscious 2–5-week-old calves, in response to stimulation of the peripheral end of the right splanchnic nerve continuously at relatively low frequencies (●; lower abscissa; from Edwards *et al.* 1980) and the corresponding higher frequency, delivered in 1 s bursts at 10 s intervals (○; upper abscissa). Vertical bars: S.E. of each mean value when in excess of the size of the symbol. Regression line calculated by the method of least squares.

DISCUSSION

Interpretation of the computed changes in adrenal vascular resistance, which occurred in these experiments, is complicated by the fact that splanchnic nerve stimulation also caused a rise in the haematocrit and presumably therefore of viscosity. However, it is unlikely that a rise in haematocrit of *ca.* 6% could account for an apparent fall in adrenal vascular resistance of *ca.* 40% (see Figs. 1 and 4) and the time courses of the two responses were also quite different. It therefore seems that splanchnic nerve stimulation under these conditions produces a substantial fall in adrenal vascular resistance. This finding was unexpected in view of the vasoconstrictor properties of catecholamines at the high concentrations at which they must have been released within the gland. This fall in vascular resistance might in part represent a passive response to the rise in perfusion pressure. However, VIP is also released from the adrenal medulla during stimulation of the splanchnic nerve in conscious calves (S. R. Bloom & A. V. Edwards, unpublished observations). The amounts are far too small for the peptide to exert any peripheral effect but the concentration in the adrenal venous effluent plasma rises to about that observed in the venous effluent from the submaxillary gland of the cat during stimulation of the chorda tympani (Bloom & Edwards, 1980; Lundberg, Ånggård, Fahrenkrug, Hökfelt & Mutt, 1980; Uddman, Fahrenkrug, Malm, Alumets, Håkanson & Sundler, 1980). The fact that VIP is such a potent vasodilator agent, and that its release by the submaxillary gland is now known to account for the vasodilatation that occurs during chorda stimulation in atropinized cats (Bloom & Edwards, 1980; Lundberg *et al.* 1980; Uddman *et al.* 1980), suggests that it may well contribute to the adrenal vasodilator responses to splanchnic nerve stimulation.

The output of both adrenaline and noradrenaline from the adrenal medulla is maximal in response to continuous stimulation of the splanchnic innervation, in the conscious calf, at 15 Hz (Edwards *et al.* 1980) whereas the optimum frequencies in bursts are 100 Hz (adrenaline) and 40 Hz (noradrenaline). In this respect the release of these amines resembles both the release of VIP from the submaxillary gland of the atropinized cat, together with the consequential fall in vascular resistance, in response to stimulation of the chorda tympani (Andersson *et al.* 1981*a*, *b*), and the fall in colonic vascular resistance which occurs in response to stimulation of the pelvic nerve in the same species (Andersson & Järhult, 1981). Presumably stimulation of the efferent innervation for 1 s at 10 s intervals causes the release of substantially less transmitter than would occur if the same frequency were employed continuously: 10% if it so happened that transmitter release was linearly related to the total number of stimuli delivered in unit time. If the amount of transmitter that the nerve terminals are capable of releasing exceeds the amount required in order to elicit a maximal response from the effector tissue, it follows that the receptor sites would become saturated, during continuous stimulation, at frequencies well below those at which the efferent nerve fibres are capable of conducting impulses. Thus, when the total amount of transmitter released in unit time is substantially reduced by the device of intermittent stimulation, the activity of the effector tissue reflects that of the efferent innervation over a much wider range of stimulus frequency. The veracity of this explanation receives strong support from Rosenblueth's observation that, even

after cutting the majority of the efferent nerve fibres, the maximal response of the effector tissue can still be approached provided the stimulus frequency is increased sufficiently (von Euler, 1959).

The fact that maximal autonomic responses occur at relatively low frequencies in response to continuous stimulation (generally of the whole innervation to a particular tissue and in anaesthetized animals) has led to the widespread belief that high stimulus frequencies are in some way unphysiological. This may or may not be true, but the evidence at present available provides an inadequate basis for any definition of the normal physiological pattern or frequency of activity in these nerve fibres. Niijima (1975) recorded from a single adrenal nerve fibre in an anaesthetized rabbit and found that it discharged irregularly at rates up to 12 Hz but the extent to which the rates of discharge of these fibres is depressed by anaesthesia is not known. In conscious human subjects post-ganglionic nerve fibres in the skin discharge naturally in *bursts*, which are unrelated to pulsatile cardiovascular events (Hagbarth, Hallin, Hongell, Torebjörk & Wallin, 1972; Delius, Hagbarth, Hongell & Wallin, 1972; Hallin & Torebjörk, 1974). The present results show that if preganglionic adrenal fibres ever fire intermittently at high frequencies in the normal animal the release of adrenaline will be enhanced preferentially.

Changes in the ratio of adrenaline to noradrenaline release from the adrenal medulla, in response to splanchnic nerve stimulation with changes in stimulus frequency, have been reported to occur in the dog by several workers using continuous stimulation (Rapela & Covian, 1954; Klepping, 1956; Rapela, 1956; Malmejac, Neverre, Bianchi & Bonnet, 1957). In this species the proportion of noradrenaline appeared to increase with increasing stimulus frequency but the changes were comparatively small and interpretation of the results is complicated by the fact that the experiments were carried out under anaesthesia, which substantially modifies the ratio in which the two amines are released in response to stimulation of the splanchnic nerve (Edwards *et al.* 1980). The present results provide clear evidence that, when the stimuli are delivered in bursts, adrenaline release is enhanced preferentially with increasing stimulus frequency over the range 2–100 Hz. Furthermore, they show that over the range 2–40 Hz significantly greater amounts of adrenaline, but not noradrenaline, are released when the same total number of stimuli are delivered in bursts. In certain species, the best documented of which is the cat, the proportions of adrenaline and noradrenaline have been found to vary with the particular type of stimulus employed (von Euler, 1956). Thus, adrenaline is released preferentially in response to hypoglycaemia in cats (Dunér, 1954) and sheep (Crone, 1965). In the cat von Euler & Folkow (1953) originally showed that carotid occlusion favours the release of noradrenaline whereas stimulation of either the brachial or sciatic plexus favours the release of noradrenaline. The effect of carotid occlusion is in accord with more recent findings that in cats, but not dogs, reduction of the pressure in the carotid sinus preferentially releases noradrenaline whereas stimulation of the carotid chemoreceptors preferentially releases adrenaline (Anichkov, Malyghina, Poskalenko & Ryzhenkov, 1960; Critchley, Ellis & Ungar, 1980), as does asphyxia (Redgate & Gelhorn, 1953). This difference between the adrenal medullary responses of cats and dogs is further exemplified by the finding that the relative proportions of the two amines that are secreted in response to electrical stimulation of the hypothalamus

vary in accordance with the position of the stimulating electrode in cats but not in dogs (Redgate & Gelhorn, 1953; Folkow & von Euler, 1954; Goldfien & Ganong, 1962; Malmejac, 1964).

Differential release of one or other of these catecholamines from the adrenal medulla in response to such stimuli as hypoglycaemia or asphyxia could well be due to differential sensitivity of the chromaffin cells themselves, to a lack of glucose or oxygen, or to an excess of carbon dioxide. It is more difficult to understand how differential release in response to electrical stimulation of the hypothalamus could be mediated peripherally. The results of the present study show that changes in the proportions of the two amines that are released from the gland, in response to direct stimulation of the whole of the efferent innervation, can be brought about simply by altering the pattern of stimulation. This finding obviates the necessity to invoke separate efferent pathways to the separate types of chromaffin cell (Palkama, 1962, 1964), although the existence of separate pathways remains an open question.

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REFERENCES

ANDERSSON, P-O., BLOOM, S. R., EDWARDS, A. V. & JÄRHULT, J. (1981*a*). Release of vasoactive intestinal peptide (VIP) from the submaxillary gland of the cat. *J. Physiol.* **313**, 20–21*P*.

ANDERSSON, P-O., BLOOM, S. R., EDWARDS, A. V. & JÄRHULT, J. (1981*b*). Effects of stimulation of the chorda tympani in bursts on submaxillary responses in the cat. *J. Physiol.* **322**, 469–483.

ANDERSSON, P-O., & JÄRHULT, J. (1981). Separation of colonic motor and blood flow responses to pelvic nerve stimulation in the cat. *Acta physiol. scand.* **113**, 263–265.

ANICHKOV, S. V., MALYGHINA, E. I., POSKALENKO, A. N. & RYZHENKOV, V. E. (1960). Reflexes from carotid bodies upon the adrenals. *Archs. int. Pharmacodyn. Thér.* **129**, 156–165.

BLOOM, S. R. & EDWARDS, A. V. (1980). Vasoactive intestinal peptide in relation to vasodilatation in the submaxillary gland of the cat. *J. Physiol.* **300**, 41–53.

BLOOM, S. R., EDWARDS, A. V., HARDY, R. N., MALINOWSKA, K. W. & SILVER, M. (1975). Endocrine responses to insulin hypoglycaemia in the young calf. *J. Physiol.* **244**, 783–803.

CRITCHLEY, J. A. J. H., ELLIS, P. & UNGAR, A. (1980). The reflex release of adrenaline and noradrenaline from the adrenal glands of cats and dogs. *J. Physiol.* **298**, 71–78.

CRONE, C. (1965). The secretion of adrenal medullary hormones during hypoglycaemia in intact, decerebrate and spinal sheep. *Acta physiol. scand.* **63**, 213–224.

DELIUS, W., HAGBARTH, K-E., HONGELL, A. & WALLIN, B. G. (1972). Maneouvres affecting sympathetic outflow in human skin nerves. *Acta. physiol. scand.* **84**, 177–186.

DUNÉR, H. (1954). The effect of insulin hypoglycaemia on the secretion of adrenaline and noradrenaline from the suprarenal of the cat. *Acta physiol. scand.* **32**, 63–68.

EDWARDS, A. V. (1981). Adrenal medullary responses to stimulation of the splanchnic nerve in bursts in the conscious calf. *J. Physiol.* **317**, 41–42*P*.

EDWARDS, A. V., FURNESS, P. N. & HELLE, K. B. (1980). Adrenal medullary responses to stimulation of the splanchnic nerve in the conscious calf. *J. Physiol.* **308**, 15–27.

EDWARDS, A. V., HARDY, R. N. & MALINOWSKA, K. W. (1974). The effects of infusions of synthetic adrenocorticotrophin in the conscious calf. *J. Physiol.* **239**, 477–498.

EULER, U. S. VON (1956). *Noradrenaline*. Springfield, Illinois: Thomas.

EULER, U. S. VON (1959). Autonomic neuroeffector transmission. In *Handbook of Physiology* sect. 1, vol. 1, ed. FIELD, J., MAGOUN, H. W. & HALL, V. E., pp. 215–238. American Physiological Society: Washington DC.

EULER, U. S. VON & FOLKOW, B. (1953). Einfluss verschiedener afferenter Nervenreize auf die

Zusammensetzung des Nebennierenmarkinkretes bei der Katze. *Arch. exp. Path. Pharmak.* **219**, 242–247.

EULER, U. S. VON & FLODING, I. (1955). A fluorimetric micromethod for differential estimation of adrenaline and noradrenaline. *Acta. physiol. scand.* **33**, Suppl. 118, 45–56.

FOLKOW, B. & EULER, U. S. VON (1954). Selective activation of noradrenaline and adrenaline producing cells in the cats' adrenal gland by hypothalamic stimulation. *Circulation Res.* **2**, 191–195.

GOLDFIEN, A. & GANONG, W. F. (1962). Adrenal medullary and adrenal cortical response to stimulation of diencephalon. *Am. J. Physiol.* **202**, 205–211.

HAGBARTH, K-E., HALLIN, R. G., HONGELL, A., TOREBJÖRK, H. E. & WALLIN, B. G. (1972). General characteristics of sympathetic activity in human skin nerves. *Acta. physiol. scand.* **84**, 164–176.

HALLIN, R. G. & TOREBJÖRK, H. E. (1974). Single unit sympathetic activity in human skin nerves during rest and various manoevres. *Acta. physiol. scand.* **92**, 303–317.

KLEPPING, J. (1956). Modalités secretoires de la médullosurrénale en fonction de la fréquence de stimulation du nerf splanchnique. *C.r. Seanc. Soc. Biol.* **150**, 705–708.

LUNDBERG, J. M., ÄNGGÅRD, A., FAHRENKRUG, J., HÖKFELT, T. & MUTT, V. (1980). Vasoactive intestinal polypeptide in cholinergic neurones of exocrine glands: functional significance of coexisting transmitters for vasodilatation and secretion. *Proc. natn. Acad. Sci.* **77**, 1651–1655.

MALMEJAC, J. (1964). Activity of the adrenal medulla and its regulation *Physiol. Rev.* **44**, 186–218.

MALMEJAC, J., NEVERRE, G., BIANCHI, M. & BONNET, D. (1957). Discussion sur la sécrétion de noradrénaline par la glande surrénale de chien. *J. Physiol., Paris* **49**, 295–299.

NIIJIMA, A. (1975). The effect of glucose on the activity of the adrenal nerve and pancreatic branch of the vagus nerve in the rabbit. *Neurosci. Lett.* **1**, 159–162.

PALKAMA, A. (1962). Distribution of adrenaline, noradrenaline, acid phosphatase, cholinesterases, and nonspecific esterases in the adrenal medulla of some mammals. *Annls Med. exp. Biol. Fenn.* **40**, Suppl. 3.

PALKAMA, A. (1964). The distribution of catecholamines and cholinesterases in the adrenal medulla. *J. Physiol.* **175**, 13–14*P*

RAPELA, C. E. (1956). Differential secretion of adrenaline and noradrenaline. *Acta. physiol. latinoam.* **6**, 1–14.

RAPELA, C. E. & COVIAN, M. R. (1954). Fréquence de stimulation des nerfs splanchnique et sécrétion surrénale d'adrénaline et de noradrénaline *C.r. Seanc. Soc. Biol.* **148**, 1667–1669.

REDGATE, E. S. & GELLHORN, E. (1953). Nature of sympathetico-adrenal discharge under conditions of excitation of central autonomic structures. *Am. J. Physiol.* **174**, 475–480.

SNEDECOR, G. W. & COCHRAN, W. G. (1967). *Statistical Methods.* Ames: Iowa State College Press.

UDDMAN, R., FAHRENKRUG, J., MALM, L., ALUMETS, J., HÅKANSON, R. & SUNDLER, F. (1980). Neuronal VIP in salivary glands: distribution and release. *Acta. physiol. scand.* **110**, 31–38.