0014-4754/94/100931-05$1.50 + 0.20

Muscarinic involvement in vascular and adrenal medullary responses to splanchnic nerve stimulation in conscious calves

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Received 18 February 1994; accepted 8 July 1994

Abstract. Stimulation of the peripheral end of the right splanchnic nerve (4 Hz for 10 min) in the presence of hexamethonium caused a small but significant rise in mean aortic blood pressure which was subsequently abolished by atropine. There were also small but significant increases in the outputs of catecholamines, [Met^5]-enkephalins and corticotrophin releasing factor (CRF) from the right adrenal gland. The catecholamine response was roughly halved after atropine while the outputs of enkephalins and CRF were unaffected. It is concluded that splanchnic sympathetic postganglionic neurones supplying the vasculature are completely blocked by cholinergic blockade whereas adrenal medullary responses persist in an attenuated form.
Key words. Sympathetic; adrenal medulla; nicotinic; muscarinic; catecholamines; enkephalins; corticotrophin releasing factor (CRF).

Adrenal medullary responses to electrical stimulation of the splanchnic sympathetic innervation are due mainly to activation of nicotinic receptors and so are greatly reduced following ganglionic blockade[1–4]. However, a minor involvement of muscarinic receptors was recognised at an early stage[1] and recent studies in the conscious calf have shown that both secretion of cortisol from the adrenal cortex and the output of catecholamines, enkephalines and corticotrophin releasing factor (CRF) from the adrenal medulla are potently stimulated by exogenous acetylcholine acting mainly via muscarinic receptors[5,6]. Even so, atropine has no effect on the adrenal steroidogenic response to splanchnic nerve stimulation, which occurs in the presence of ACTH, and relatively little effect on the adrenal medullary responses[4]. This situation favours the suggestion that the nicotinic receptors are situated mainly at synapses on chromaffin cell membranes while muscarinic receptors are located elsewhere on the surface of the chromaffin cells[7]. Potentiation of steroidogenesis in the adrenal cortex, during splanchnic nerve stimulation, is thought to be due to activation of vasoactive intestinal peptide (VIP)-containing fibres both in conscious calves and perfused pig adrenal glands[8,9]. VIP is released from the adrenal gland during stimulation of the splanchnic nerves[10] and intra-aortic infusions of a low dose of VIP cause a small but significant increase in catecholamine output in this species[11]. Furthermore, VIP has been reported to act as a transmitter in the adrenal medulla of the rat[12], where it is reportedly blocked by naloxone[13]. The present study was undertaken to investigate non-nicotinic adrenal medullary responses to splanchnic nerve stimulation in conscious calves, pretreated with hexamethonium, by assessing the effects of naloxone and atropine.

Materials and methods

The effects of electrical stimulation of the peripheral end of the right splanchnic nerve (4 Hz continuously for 10 min) were investigated in five conscious pedigree Jersey calves (15–37 days old; 26.0–34.4 kg b. wt). A standard 20–30 V square wave stimulus (pulse width 0.5 msec) was employed and was invariably below behavioural threshold. Each animal had previously been fitted with an adrenal clamp, intravascular catheters and a fluid electrode had been attached to the peripheral end of the right splanchnic nerve[14,15] under general halothane anaesthesia. Experiments were carried out 3–4 h after surgery, during which time the animals had made a full recovery from anaesthesia. Aortic blood pressure and heart rate were monitored continuously by means of a Devices M19 recorder. Right adrenal blood flow was estimated gravimetrically and adrenal vascular resistance estimated by dividing the perfusion pressure (mean aortic blood pressure) by right adrenal blood flow. Animals were pretreated with hexamethonium (Hexamethonium bromide, Sigma, 25.0 pmol.kg^{-1} i.v. and then 12.5 pmol.kg^{-1} at 30 min intervals) and also given naloxone (Naloxone hydrochloride, Sigma, 5.5 nmol.kg^{-1} i.v. at 30 min intervals as required) and atropine (atropine methyl nitrate, Sigma, 0.35 μmol.kg^{-1} i.v.). Samples of arterial blood were collected at intervals into heparinized tubes containing phenylmethylsuphonyl fluoride (PMSF, final concentration 0.1 mM, Sigma) for haematocrit and

glucose estimations and samples of adrenal venous effluent blood collected in the same way for [Met^5]-enkephalin and CRF estimations and into tubes containing ca 10 µmol ethylenediamintetraacetic acid (EDTA, Sigma) for catecholamine estimations. Cardiovascular responses to i.v. infusions of exogenous adrenaline and noradrenaline were determined in a separate group of 6 conscious calves in which intravascular catheters had previously been implanted under general halothane anaesthesia.

Epinephrine and norepinephrine were measured by HPLC with electrochemical detection[16]. CRF was determined by radioimmunoassay, essentially as described by Vale and colleagues[17] and [Met^5]-enkephalin was measured by a specific radioimmunoassay as described previously[18,19]. Results are expressed as mean ±SEM. Statistical significance was assessed by Student's *t* test except where otherwise stated.

Results and discussion

Stimulation of the peripheral end of the right splanchnic nerve at 4 Hz for 10 min produced a small but significant rise in mean aortic blood pressure in 5 conscious calves pretreated with hexamethonium (25.0 µmol.kg^{-1} i.v.; fig. 1). This amounted to a rise from 71 ± 5 mm Hg at time = 0 to a peak value of 83 ± 3 mm Hg at 2.5 min ($p < 0.001$; paired *t* test). Following the additional administration of naloxone (5.5 nmol.kg^{-1} i.v.) the hypertensive response to splanchnic nerve stimulation was slightly greater with the mean pressure rising from 73 ± 4 to 88 ± 1 mm Hg ($p < 0.01$) over the same period. It was then abolished by subsequent additional administration of atropine (0.35 nmol.kg^{-1} i.v.; fig. 1). In contrast there was no significant change in mean heart rate in response to splanchnic nerve stimulation following hexamethonium, with or without naloxone and atropine.

Stimulation of the peripheral end of the splanchnic nerve (4 Hz for 10 min) also produced small but significant increases in right adrenal catecholamine, [Met^5]-enkephalin and CRF output (figs 2–4). Unlike the response to splanchnic nerve stimulation in the absence of hexamethonium[10] the mean peak output of epinephrine at 2.5 min (45 ± 5 pmol.min^{-1}.kg^{-1} was significantly greater than that of norepinephrine (29 ± 4 pmol.min^{-1}.kg^{-1}; $p < 0.05$). Neither response was affected by naloxone and the output of both was roughly halved after atropine (to 29 ± 4 and 18 ± 2 pmol.min^{-1}.kg^{-1} at 2.5 min respectively; $p < 0.25$). In contrast, neither the [Met^5]-enkephalin nor the CRF responses to splanchnic nerve stimulation were significantly reduced by either naloxone or atropine (figs 3 and 4).

The extent to which the release of catecholamines from the adrenal gland could have contributed to the hypertensive response was investigated in a separate group of

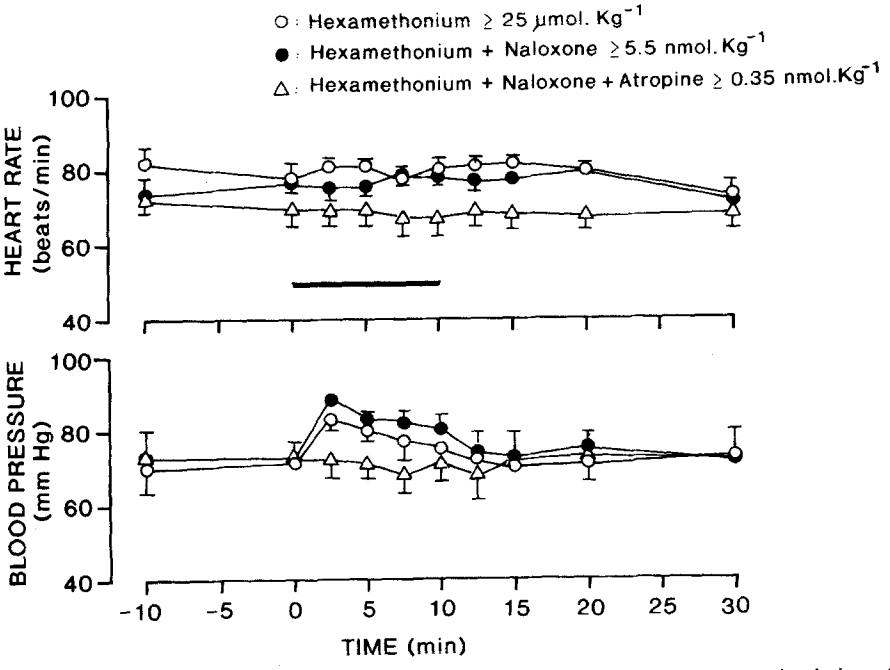


Figure 1. Changes in mean heart rate and aortic blood pressure in 5 conscious calves in response to stimulation of the peripheral end of the right splanchnic nerve (4 Hz for 10 min) in the presence of hexamethonium, with and without naloxone and atropine. Mean values ±SEM.

Figure 3. Changes in mean right adrenal [Met^5]-enkephalin output in 5 conscious calves in response to stimulation of the peripheral end of the right splanchnic nerve (4 Hz for 10 min) in the presence of hexamethonium, with and without naloxone and atropine. Mean values ±SEM.

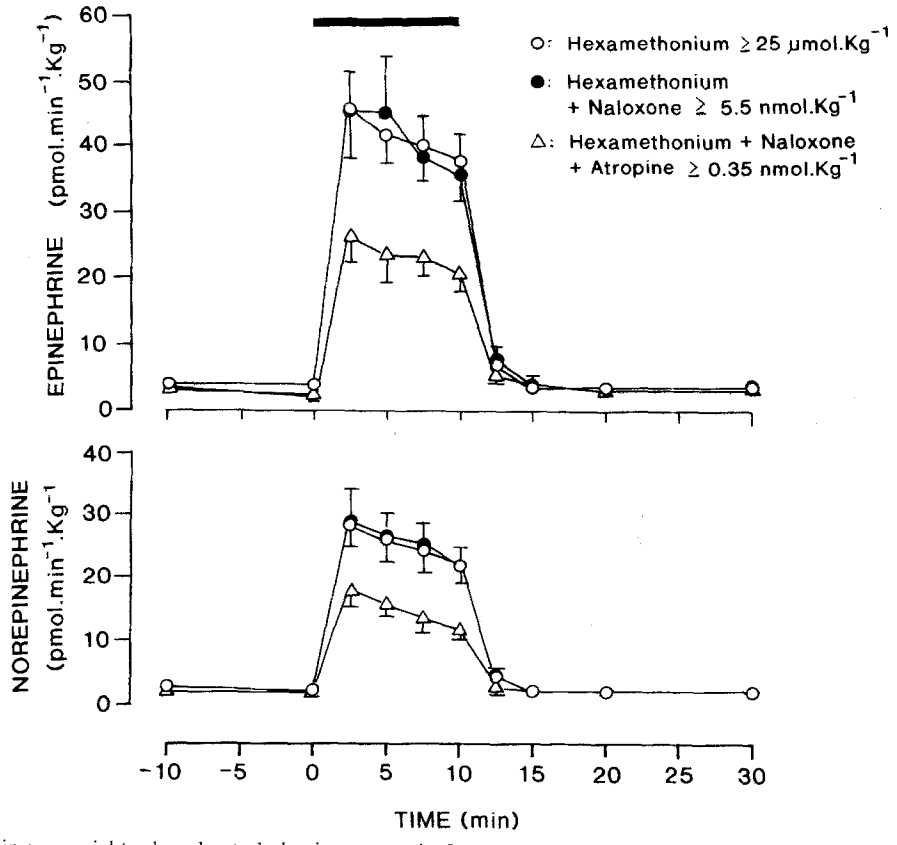
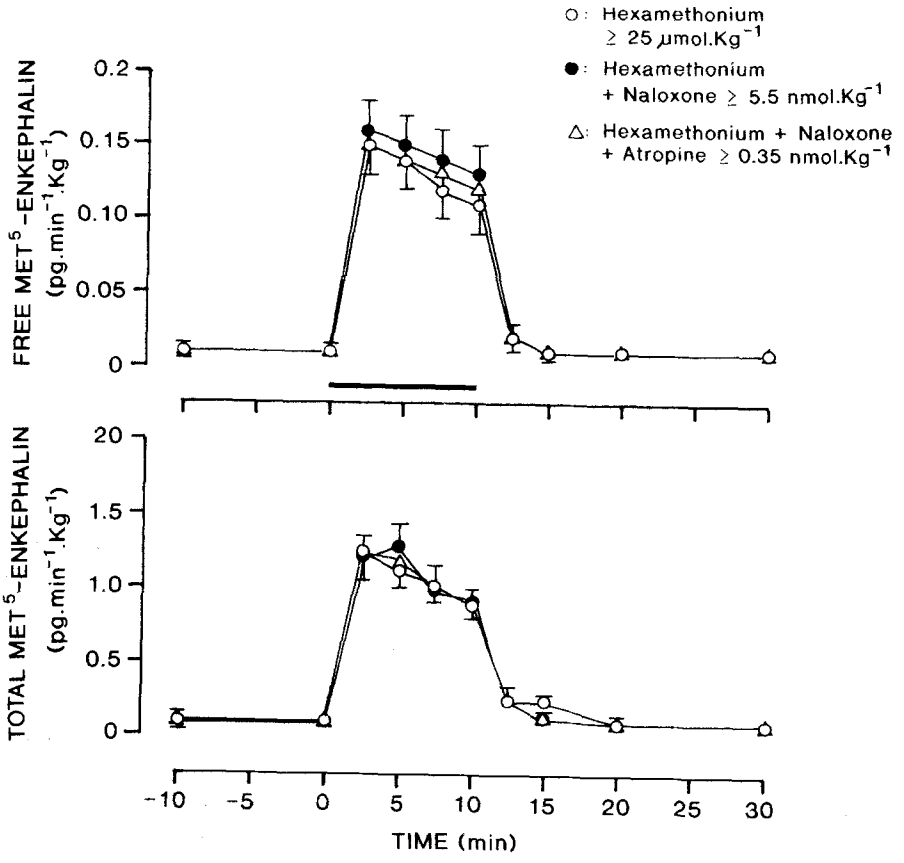


Figure 2. Changes in mean right adrenal catecholamine output in 5 conscious calves in response to stimulation of the peripheral end of the right splanchnic nerve (4 Hz for 10 min) in the presence of hexamethonium, with and without naloxone and atropine. Mean values ±SEM.



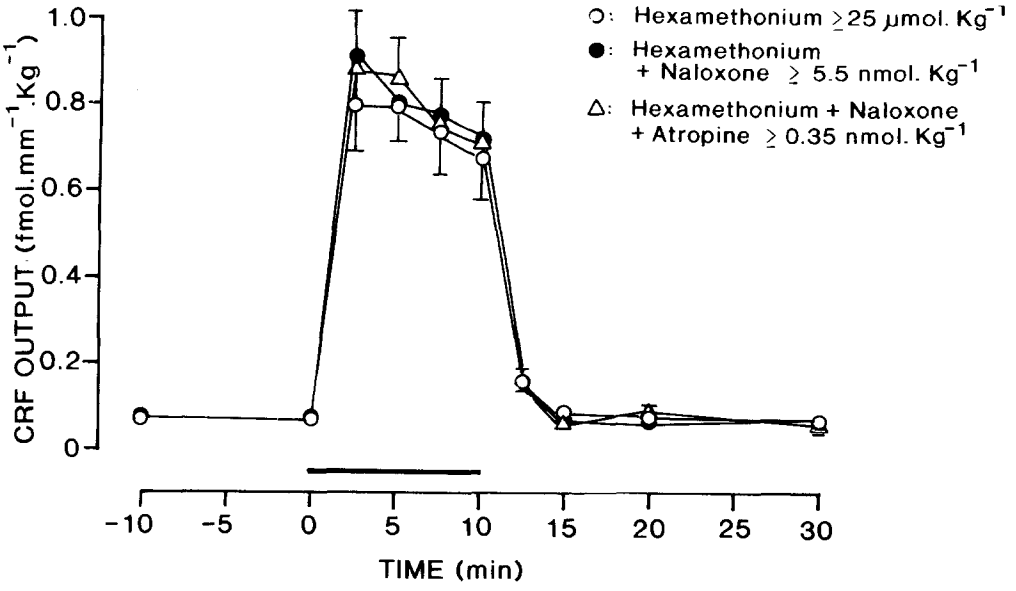


Figure 4. Changes in mean right adrenal CRF output in 5 conscious calves in response to stimulation of the peripheral end of the right splanchnic nerve (4 Hz for 10 min) in the presence of hexamethonium, with and without naloxone and atropine. Mean values ±SEM.

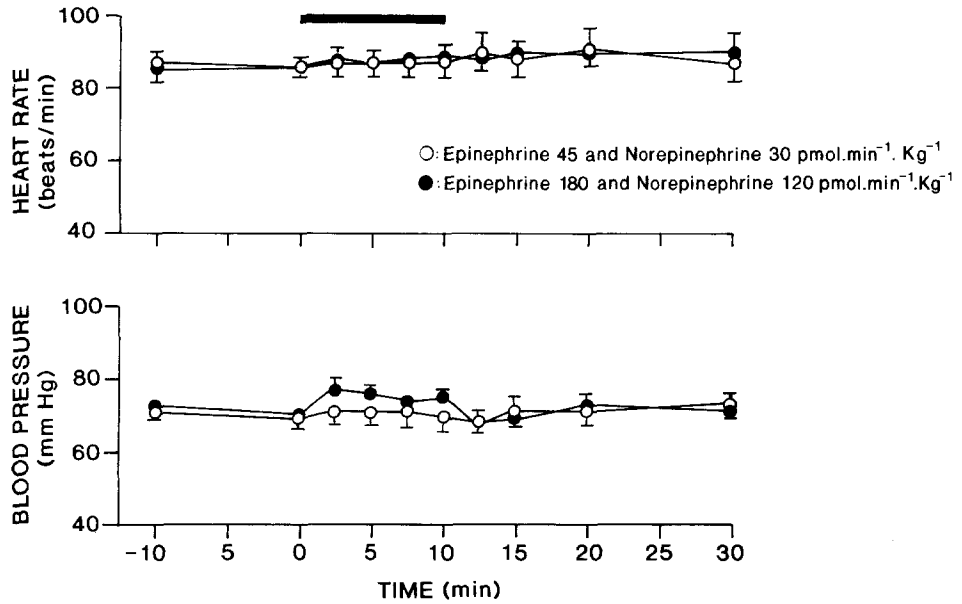


Figure 5. Changes in mean aortic blood pressure and heart rate in 4 conscious calves in response to infusions of catecholamiens in the presence of hexamethonium ($25 \geq \mu mol.kg^{-1}$). Mean values ±SEM.

4 conscious calves which were pretreated with hexamethonium and then given i.v. infusions of epinephrine and norepinephrine for the same period as with splanchnic nerve stimulation. A dose of 45 $pmol.min^{-1}.kg^{-1}$ epinephrine and 30 $pmol.min^{-1}.kg^{-1}$ norepinephrine, which precisely reproduced the maximum mean output of catecholamines during splanchnic nerve stimulation in the presence of hexamethonium (fig. 2), had no significant effect on mean aortic blood pressure. Increasing the amounts of the catecholamines fourfold produced a small but significant rise in mean aortic blood pressure. Thus the mean average value during the infusion (76 ± 1 mm Hg) then signficantly exceeded the mean average value before and after the infusion (71 ± 1 mm Hg).

After hexamethonium the blood pressure could not have been affected by the release of catecholamines from the adrenal medulla. The total amounts secreted were too small and, in any case, almost all the adrenal effluent blood was collected for analyses during the period of splanchnic nerve stimulation and so prevented from entering the circulation. It follows that the hyper-

tensive response to stimulation of the sympathetic innervation in the calf can be completely eliminated by combined nicotinic and muscarinic blockade. This indicates that, whereas ganglionic transmission is largely achieved by activation of nicotinic receptors, muscarinic receptors also contribute, but no other transmitter or receptor type is involved. In contrast, the adrenal medullary responses cannot be accounted for solely by activation of cholinergic receptors. The residual non-nicotinic catecholamine response was further significantly reduced after atropine whereas the residual non-nicotinic increase in the output of enkephalins and CRF were not significantly affected thereby. This is suggestive of different sites of release and could be explained if the CRF and the enkephalins, which are released during splanchnic nerve stimulation after hexamethonium, originate from nerve terminals within the gland whereas the catecholamines are released from the chromaffin cells. This would be consistent with the finding that enkephalins are present both in splanchnic nerve terminals and chromaffin cells[20] and with the fact that CRF is a well-recognised neuropeptide in widely spread nerve tracts in the brain[21]. In the calf, the amounts of CRF released in response to splanchnic nerve stimulation, assessed by the rise in the concentration in the arterial plasma, far exceed that released from the adrenal gland, which strongly suggests that it is indeed released from sympathetic nerve terminals[22].

Acknowledgements. This work was supported by the British Heart Foundation. It is a particular pleasure to acknowledge the skilled technical assistance provided by Mrs B. N. Daw and Mr P. M. M. Bircham.

1 Feldberg, W., Minz, B., and Tsudzimura, H., J. Physiol., Lond. *81* (1934) 286.
2 Comline, R. S., and Silver, M., J. Physiol., Lond. *156* (1961) 424.
3 Comline, R. S., and Silver, M., J. Physiol., Lond. *183* (1966) 305.
4 Edwards, A. V. and Jones C. T., J. Physiol. Lond. *459* (1993) 433P.
5 Jones, C. T., Edwards, A. V., and Bloom, S. R., J. Physiol., Lond. *439* (1991) 481.
6 Jones, C. T., and Edwards, A. V., J. Physiol., Lond. *444* (1991) 605.
7 Wakade, A. R., and Wakade, T. D., Neuroscience *10* (1983) 973.
8 Bloom, S. R., Edwards, A. V., and Jones, C. T., J. Physiol., Lond. *391* (1987) 441.
9 Erhardt-Bornstein, M., Bornstein, S. R., Scerbaum, W. A., Pfeifer, E. F., and Holst, J. J., Neuroendocrinology *54* (1991) 623.
10 Bloom, S. R., Edwards, A. V., and Jones, C. T., J. Physiol., Lond. *397* (1988) 513.
11 Edwards, A. V., and Jones, C. T., J. Physiol., Lond. *468* (1993) 515.
12 Wakade, T. D., Blank, M. A., Malhotra, R. K., Poucho, R., and Wakade, A. R., J. Physiol., Lond. *444* (1991) 349.
13 Wakade, A. R., J. Neurochem. *50* (1988) 1302.
14 Edwards, A. V., Hardy, R. N., and Malinowska, K. W., J. Physiol., Lond. *239* (1974) 477.
15 Edwards, A. V., Furness, P. N., and Helle, K. B., J. Physiol., Lond. *308* (1980) 15.
16 Arkinstall, S., and Jones, C. T., J. Reprod. Fert. *73* (1985) 547.
17 Vale, W., Vaughan, G., Yamamoto, G., Bruhn, T., Douglas, C., Dalton, D., Rivier, C., and Rivier, J., Meth. Enzym. *103* (1983) 565.
18 Edwards, A. V., Hansell, D., and Jones, C. T., J. Physiol., Lond. *379* (1986) 1.
19 Edwards, A. V., and Jones, C. T., J. Physiol., Lond. *382* (1987) 385.
20 Schultzberg, M., Lundberg, J. M., Hökfelt, T., Terenius, L., Brandt, J., Elde, R. P., and Goldstein, M., Neuroscience *3* (1978) 169.
21 Swanson, L. W., Sawchenko, P. E., Rivier, J., and Vale, W. W.W., Neuroendocrinology *36* (1983) 165.
22 Edwards, A. V., and Jones, C. T., J. Physiol. Lond. *400* (1988) 89.