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Inhibitory regulation by co-released peptides of catecholamine secretion by the canine adrenal medulla

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1 We have stimulated the peripheral end of the cut left splanchnic nerve in anaesthetized dogs while collecting the venous effluent of the left adrenal gland for catecholamine estimation.

2 With low frequency stimulation the resting output of catecholamines was inhibited but at high frequencies it was augmented.

3 The inhibition of catecholamine output by low frequency stimulation was reversed by opiate antagonists (naloxone and nalmefene) but enhanced by angiotensin converting enzyme inhibitors (captopril and enalapril).

Introduction

Opioid and other peptides are stored and released with catecholamines in the adrenal medulla (Viveros *et al.*, 1980). Inhibition of catecholamine secretion by opiates has been observed, for which both prejunctional and postjunctional mechanisms have been suggested (Viveros *et al.*, 1980; Lemaire *et al.*, 1981).

The opiate antagonist naloxone in man attenuates the hypotensive action of captopril (Rubin *et al.*, 1984), and it was inferred that captopril acts partly by inhibiting the enzymatic degradation of endogenous opioid peptides.

Electrical stimulation of the peripheral end of the cut left splanchnic nerve in the dog, at low frequencies in the presence of captopril, inhibits catecholamine secretion from the left adrenal gland (Maclean & Ungar, 1986).

By raising the carotid sinus pressure in anaesthetized dogs the output of catecholamines from the adrenal medulla can be reduced to low levels, much lower than in similarly anaesthetized dogs with denervated adrenal glands (Maclean & Ungar, 1986). From this low resting level, infusion of naloxone induced a 5 fold increase in resting catecholamine output and also enhanced the increase in output when the carotid sinus pressure was lowered (Maclean & Ungar, 1985).

The aim of this work was to explore the possibility that these phenomena reflect a tonic inhibition of catecholamine release by endogenous opioid peptides. A preliminary account of part of the work has been published (Maclean & Ungar, 1985).

Methods

Anaesthesia, respiration and temperature

Mongrel dogs of either sex, weighing 16–25 kg were anaesthetized with an i.v. injection of sodium pentobarbitone (30 mg kg^{-1}) and anaesthesia was maintained by infusion at a rate of approximately one-tenth of the initial dose per hour, adjusted so as to suppress the paw-withdrawal reflex.

The trachea was cannulated and connected to a Starling 'Ideal' pump, and the lungs ventilated with a metered oxygen-nitrogen mixture. Arterial blood samples were collected at intervals and pH and blood gas tensions measured on a Radiometer BMS3 analyser. Pa_{CO_2} was held at 5 kPa and Pa_{O_2} above 20 kPa. pH was adjusted to 7.4 by injection of an appropriate volume of molar sodium bicarbonate solution.

Body temperature was held near 37°C by a heating pad linked to a rectal thermistor probe.

Collection and analysis of adrenal venous blood

The left adrenolumbar vein was cannulated towards the gland for the collection of the adrenal venous outflow, and the adrenal vein was ligated between the gland and the vena cava. Samples of the outflow were collected for 1 min periods in cooled graduated tubes, and centrifuged for 10 min at 1000 *g*. The volumes of plasma and packed cells were recorded, and the plasma separated for estimation of catecholamines (CA) by high performance liquid chromatography, as described by Maclean & Ungar (1986). Throughout

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this paper we refer to total CA, by which we mean the sum of adrenaline and noradrenaline. The percentage of adrenaline in resting and evoked release of CA was always in the range 80–85%.

Denervation and electrical stimulation of left adrenal gland

The left splanchnic nerve was dissected free and its central roots were ligated and cut. An arc of tissue was crushed medially and rostrally from the nerve, to eliminate adrenal sympathetic fibres not carried by the splanchnic trunk. This procedure abolishes the baroreceptor reflex response of the adrenal medulla (Critchley *et al.*, 1982). The splanchnic nerve was placed on a pair of platinum electrodes and stimulated with pulses of 10 V amplitude and 2 ms duration.

Inhibition of angiotensin converting enzyme (ACE)

Captopril or enalapril (1 mg kg^{-1}) was injected i.v. This dose of either drug was found to give full inhibition of ACE for the duration of the experiment, as assessed by the abolition of the pressor response to angiotensin I (Maclean & Ungar, 1986); with enalapril almost an hour was needed for the full effect to develop.

Analysis of results

The statistical significance of results was assessed by the paired *t* test. Unless otherwise stated, all changes discussed are significant ($P < 0.05$).

Drugs

The following drugs were used (sources in parentheses): sodium pentobarbitone (May & Baker), heparin (Weddel Pharmaceuticals), captopril (Squibb), enalapril (Merck, Sharpe & Dohme), naloxone (Sigma) and nalmefene (Key Pharmaceuticals).

Results

Responses to splanchnic nerve stimulation

In 14 dogs the peripheral end of the cut splanchnic nerve was stimulated at a range of frequencies from 0.625 to 20 pulses s^{-1} (Hz). The results are illustrated in Figure 1. At 5, 10 and 20 Hz, frequency-dependent stimulation of CA release was seen, but at 1.25 Hz there was a significant inhibition of CA release. The mean response to stimulation at 0.625 and at 2.5 Hz did not show significant stimulation or inhibition.

Effects of opiate antagonists

In 4 dogs stimulation at 10 Hz was repeated during i.v. infusion of naloxone (15 $\mu g\,kg^{-1}\,min^{-1}$). The mean resting output of CA from the gland rose from 24 to 152 pmol $min^{-1}\,kg^{-1}$, and the mean increment in output during splanchnic nerve stimulation rose from 50 to 117 pmol $min^{-1}\,kg^{-1}$.

In another 5 dogs the splanchnic nerve was stimulated at a range of frequencies before and after injection of the long-acting opiate antagonist nalmefene (100 $\mu g\,kg^{-1}$, i.v.). The mean resting output of CA from the gland rose from 45 ± 9 to 92 ± 20 pmol $min^{-1}\,kg^{-1}$. The results are illustrated in Figure 2. The inhibition of release previously seen with low-frequency stimulation was now reversed, and significant, frequency-dependent stimulation of catecholamine release was seen from 0.625 to 10 Hz.

Effects of angiotensin converting enzyme inhibitors

In 6 dogs the splanchnic nerve was stimulated at a range of frequencies before and after injection of enalapril (1 mg kg^{-1}, i.v.). The mean resting output of CA from the gland was 25.8 ± 2.7 pmol $min^{-1}\,kg^{-1}$ before, and 21.8 ± 6.7 pmol $min^{-1}\,kg^{-1}$ after the injection of enalapril. The results are illustrated in Figure 3.

The inhibition of release by stimulation at 1.25 Hz was not significantly affected by enalapril. The stimulation of release at 5 Hz was abolished, and that at 10 Hz much reduced by enalapril.

In all of these dogs, nalmefene (100 $\mu g\,kg^{-1}$, i.v.) was then injected, and stimulation repeated at 1.25, 5 and

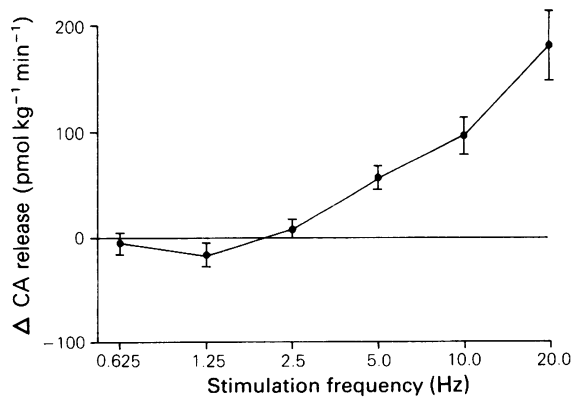

Figure 1 Increments in catecholamine (CA) release from the left adrenal gland during stimulation of the peripheral cut end of the left splanchnic nerve at 6 frequencies. Mean results from 14 dogs. The vertical bars represent s.e.mean values.

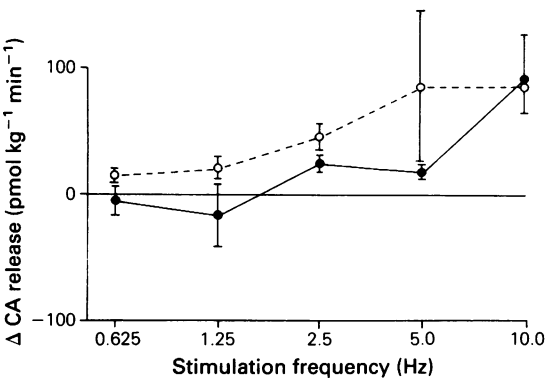

Figure 2 Increments in catecholamine (CA) release from the left adrenal gland during stimulation of the peripheral cut end of the left splanchnic nerve at 5 frequencies. Mean results from 5 dogs: (●) represent results before, and (○) those after, the injection of nalmefene. The vertical bars represent s.e.mean values.

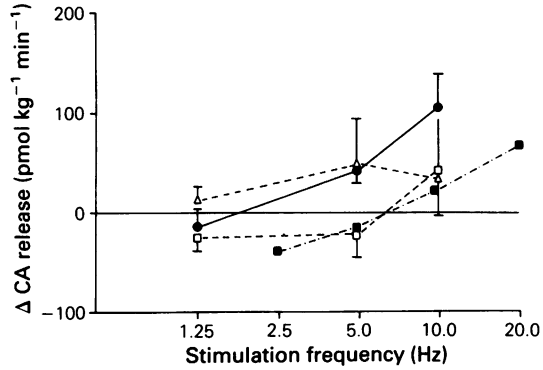

Figure 3 Increments in catecholamine (CA) release from the left adrenal gland during stimulation of the peripheral cut end of the left splanchnic nerve at 3 frequencies. Mean results from 6 dogs: (●) represent results before drugs; (□) those after injection of enalapril; (△) results after both enalapril and nalmefene; (■) represents results with captopril (obtained under similar conditions) from Maclean & Ungar (1986). The vertical bars represent s.e.mean values.

10 Hz. The results are also shown in Figure 3. At 1.25 Hz inhibition was reversed to excitation, giving release of CA of the same order of magnitude as seen with nalmefene alone. At 5 Hz release of CA was restored to the level before suppression by enalapril.

These results with enalapril closely resemble those previously obtained with captopril, under similar conditions, by Maclean & Ungar (1986), also illustrated in Figure 3.

Discussion

In anaesthetized dogs a denervated adrenal gland has quite a high resting output of CA. We have previously shown that this can be explained by blood-borne secretagogues, including corticosteroids and angiotensin II (AII) (Critchley *et al.*, 1982; Maclean & Ungar, 1986). We have now shown that this resting output is inhibited by electrical stimulation of the splanchnic nerve at low frequencies, and augmented only by high frequency stimulation. This inhibition of CA release by low frequency electrical stimulation is reversed by naloxone or nalmefene to an excitation of release at all frequencies of stimulation. This suggests either an inhibitory component in the splanchnic innervation of the gland, or inhibitory co-transmission, involving an inhibitory transmitter acting on opiate, or related peptide, receptors. We previously found that naloxone induced much larger increases in CA output from adrenal glands with intact innervation, suggesting that the inhibitory process may be tonically active (Maclean & Ungar, 1985).

The mutual antagonism of opiate antagonists and ACE inhibitors in their effects on catecholamine release has two possible explanations:

(i) The evoked release of CA in response to nerve stimulation is dependent on circulating AII, and in the absence of AII the inhibitory effects of co-released peptides at low frequencies of nerve stimulation are unmasked.

(ii) Blockade by ACE inhibitors of the enzymatic degradation of other endogenous peptides that exert inhibitory regulation of CA release.

We think that circulating AII may be the more important, because small amounts of exogenous AII restore the output of CA after ACE inhibition (Maclean & Ungar, 1986). Also, the effects of opiate antagonists are pronounced at low frequencies of stimulation, in contrast with the ACE inhibitors which are more effective at high frequencies.

Our results fit a model in which both resting output of CA and neurally evoked release are regulated by a balance of excitatory and inhibitory modulators, including steroids, AII and opioid peptides.

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