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THE EFFECT OF DIFFERENT CALCIUM CONCENTRATIONS ON THE INHIBITORY EFFECT OF PRESYNAPTIC α-ADRENOCEPTORS IN THE RAT VAS DEFERENS

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The effects of different calcium concentrations on the twitch-inhibitory potency of clonidine and of guanethidine have been examined in the rat isolated vas deferens. Vasa deferentia did not respond to field stimulation at 0.3 Hz in Krebs solution containing 0.325 mmol/l of calcium. Increasing the calcium concentration to 5.2 mmol/l caused a concentration-dependent increase in size of the twitch response. The twitch-inhibitory potency of clonidine was inversely proportional to the calcium concentration. The inhibitory effect of guanethidine was not influenced by changes in calcium concentration. The results show that the modulation of motor function by presynaptic α-adrenoceptors in the rat vas deferens is calcium-dependent.

Introduction The release of noradrenaline from sympathetic nerves during electrical stimulation is inhibited by exogenously administered α-adrenoceptor agonists and enhanced by α-adrenoceptor antagonists. These compounds exert their effects at α-adrenoceptors located presynaptically on the sympathetic nerve terminal (Langer, 1977; Starke, 1977). In contrast, the tyramine-induced release of noradrenaline is unaffected by α-adrenoceptor agonists or antagonists (Starke & Montel, 1974). Tyramine and nerve stimulation-induced noradrenaline release differ in that tyramine-induced release is not calcium-dependent (Lindmar, Loffelholz & Muscholl, 1967). Accordingly, it has been suggested that stimulation of the presynaptic α-adrenoceptors somehow reduces the accumulation of intraneuronal free calcium and thus prevents the electrosecretory coupling process (Stjärne, 1973; Starke & Montel, 1974). Langer, Dubocovich & Celuch (1975) confirmed this suggestion by demonstrating that the presynaptic effect of noradrenaline in the perfused cat spleen was more marked when the calcium concentration of the perfusate was reduced.

Low frequency field stimulation of the rat isolated vas deferens produces individual twitch responses that are reduced by clonidine and guanethidine. Both drugs act presynaptically to reduce the release of the motor transmitter. Clonidine exerts its twitch inhibitory effect by stimulating presynaptic α-adrenoceptors (Vizi, Somogyi, Hadhazy & Knoll, 1973) and this effect can be reversed by α-adrenoceptor antagonists (Drew, 1977). Guanethidine, on the other hand, exerts a neurone blocking action (Ambache & Aboo Zar, 1971; Swedin, 1971) which is not reversed by presynaptic α-adrenoceptor blockade (Drew, 1977). In the experiments described here the effects of calcium on the presynaptic α-inhibitory feedback system in the rat isolated vas deferens preparation have been examined by measurement of the twitch-inhibitory potency of clonidine and guanethidine in the presence of different calcium concentrations.

Methods Vasa deferentia were removed from rats (200 to 300 g), desheathed and suspended under 0.5 to 0.8 g tension at 37°C in Krebs solution with the following composition (mmol/l): Na^+ 143.4, K^+ 5.9, Ca^{2+} 0.325, Cl^- 122.9, $H_2PO_4^-$ 1.2, HCO_3^- 25.0 and glucose 11.1. Platinum electrodes were placed near the top and bottom of the tissue and field stimulation was carried out at 0.3 Hz with square wave pulses of 2 ms duration, delivered with a current strength of 40 mA from a Palmer 8048 stimulator. Contractions of the vasa deferentia were recorded isometrically with Dynamometer UF1 2 oz strain gauges and were displayed on a Devices chart recorder. Under these low calcium conditions there was little or no response to field stimulation and so more calcium chloride was added to the bathing fluid to raise the concentration to 0.65, 1.3, 2.6 or 5.2 mmol/l. When the size of the twitch responses produced in the presence of one of the higher calcium concentrations had become constant, a cumulative concentration-response curve to clonidine or to guanethidine was established. The concentrations of clonidine and guanethidine that reduced the twitch response by 50% at each calcium concentration were calculated graphically.

The following drugs were used: clonidine hydrochloride (Catapres-Boehringer-Ingelheim); guanethidine monosulphate (Ismelin-Ciba); piperoxan hydrochloride (May and Baker). Drug solutions were made up immediately before use in 0.9% w/v NaCl solution

Table 1 The influence of Ca^{2+} concentration on the size of the twitch response to field stimulation (0.3 Hz) of the rat isolated vas deferens and on the sensitivity to clonidine and guanethidine

Calcium concentration (mmol/l)	*n*	*Peak twitch response (mg tension ± s.e.)*	*n*	*EC_{50} clonidine* (geometric mean in nmol/l)*	*n*	*EC_{50} guanethidine (geometric mean in μmol/l)*
0.65	5	117 ± 10	5	1.8 (1.0–2.9)†		
1.30	12	472 ± 62	6	3.9 (3.0–4.8)	6	9.0 (5.5–14.7)
2.60	6	853 ± 90	6	7.5 (4.4–12.9)		
5.20	11	873 ± 160	6	14.4 (10.0–20.7)	5	6.0 (3.1–11.4)

* EC_{50} for clonidine at each calcium concentration was significantly different ($P = <0.05$) from that obtained at any other concentration.
† 95% confidence limits given in parentheses.

(saline) and concentrations in the text refer to the free base.

Results Little or no response was obtained to field stimulation of vasa deferentia suspended in Krebs solution containing 0.325 mmol/l of calcium. An increase in the calcium concentration of the Krebs solution to 0.65, 1.3, 2.6 or 5.2 mmol/l was accompanied by a concentration-dependent increase in the size of the twitch response (Table 1). The subsequent administration of clonidine caused a concentration-dependent inhibition of the twitch response. However, it was observed that the concentration-response curve to clonidine was progressively displaced to the right as the calcium concentration was increased. Thus, the twitch-inhibitory potency of clonidine was inversely proportional to the calcium concentration (Table 1). At the end of each experiment piperoxan (4.3 μmol/l) abolished the twitch inhibitory effect of clonidine, confirming that inhibition was mediated via α-adrenoceptors.

In contrast to clonidine, the twitch inhibitory effect of guanethidine was unaffected by changes in the calcium concentration (Table 1) and was not reversed by piperoxan.

Discussion The size of the twitch response to field stimulation of the rat isolated vas deferens was directly proportional to the calcium concentration of the bathing fluid. This probably reflects an increase in the intracellular calcium available for muscle contraction, because Kasuya & Goto (1968) found that the maximum contractile responses of the rat vas deferens to exogenous noradrenaline, angiotensin or potassium increased with increasing calcium concentration. Even so, an enhanced release of the motor transmitter may also contribute to the increased twitch response.

The twitch-inhibitory potency of clonidine was inversely proportional to the calcium concentration; an eight fold increase in calcium led to an eight fold increase in the EC_{50} for clonidine. Marshall, Nasmyth & Shepperson (1977) showed, in the mouse vas deferens, that the twitch-inhibitory effect of clonidine at each stimulation frequency (0.2 to 16 Hz) was enhanced when the calcium concentration of the bathing fluid was halved.

It could be argued that the reduced twitch-inhibitory potency of clonidine in the presence of increasing calcium concentration is related to the increase in size of the resting twitch response. This proposal is untenable because the twitch-inhibitory potency of guanethidine, which does not act via presynaptic α-adrenoceptors, was unaltered by an increase in calcium concentration. Wilson (1970) reported similar findings with the guinea-pig isolated vas deferens preparation.

In conclusion, these results confirm that the inhibitory effect of stimulation of presynaptic α-adrenoceptors in the rat vas deferens is calcium-dependent.

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