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Effect of physostigmine and cocaine on noradrenaline-induced contractions of the rat anococcygeus muscle

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The cholinergic innervation of the rat anococcygeus muscle constitutes about 5% of the nerve profiles (Gillespie 1980). It is doubtful if this is an important neural control since neither motor nor inhibitory responses to nerve stimulation are influenced by atropine. Gillespie & McGrath (1973) reported the origin of the motor and inhibitory nerves to be in the spinal cord with the motor being characteristically sympathetic and the inhibitory conforming anatomically to the parasympathetic division. Anticholinesterases potentiate the motor response to extrinsic nerve stimulaition but not to field stimulation (McKirdy & Muir 1978) but do not affect responses to inhibitory nerve stimulation (Gillespie 1980). Atropine reduces the effect of noradrenaline on the contractions of the muscle competitively. This antagonism was quantifiable with a pA_2 value of 6·52 (Tayo 1981). Because of these puzzling results the interactions of noradrenaline and physostigmine have been examined since the rat anococcygeus muscle has a high cholinesterase activity (Smith & Spriggs 1979). The results have also been compared with cocaine, acetylcholine and noradrenaline interactions.

Materials and methods

Male albino rats, 200–250 g were killed by a blow on the head, bled and the anococcygeus muscles removed according to Gillespie (1972) and set up in a 10 ml organ bath under a resting tension of 0·6 g; one muscle served as control. The aerated bathing fluid, maintained at 36 °C, has the following composition (mM litre^{-1}): NaCl, 137; KCl, 2·4; $CaCl_2$, 1·8; $MgCl_2$, 1·0; Na_2HPO_4, 0·2; $NaHCO_3$, 11·9 and glucose, 5·5. After equilibration, during which the bathing solution was replaced every 10 min, contractions were recorded using 5 × magnification. Noradrenaline was left to act for 90 s and acetylcholine and carbachol for 120 s. Physostigmine and cocaine were added 5 min before the agonists. There was little difference in the effect of physostigmine at 5 and at 15 min.

Results were expressed as mean ± s.e. and subjected to Student's *t*-test and differences were regarded as significant when $P < 0.05$.

The following drugs were used: (−)-noradrenaline (BDH), acetylcholine chloride (ACh) (Sigma), carbamoylcholine chloride (Carbachol, BDH), physostigmine salicylate (Burroughs-Wellcome) and cocaine hydrochloride.

Results

Noradrenaline ($1·2 \times 10^{-6}$–$3·8 \times 10^{-5}$ M) and acetylcholine ($1·1 \times 10^{-5}$–$2·8 \times 10^{-3}$ M) contracted the muscle concentration-dependently. The -log EC50 values of noradrenaline and ACh were 5·15 ± 0·05 (n = 6) and 3·40 ± 0·10 (n = 5) respectively. ACh was a much weaker agonist than noradrenaline in this tissue (Figs 1, 2).

Physostigmine (7.50×10^{-8}–$3·0 \times 10^{-7}$ M) potentiated the effects of noradrenaline in a concentration-dependent manner (Fig. 1) but failed to influence the effects of submaximal and maximal concentrations of noradrenaline. The −log EC50 value of noradrenaline was increased at every concentration of physostigmine used. Thus, the values were 5·32 ± 0·07 (n = 5), 5·50 ± 0·10 (n = 6) and 5·60 ± 0·03 (n = 5) in the presence of physostigmine $7·50 \times 10^{-8}$ M, $1·50 \times 10^{-7}$ M and $3·0 \times 10^{-7}$ M respectively. The increase produced by physostigmine $7·50 \times 10^{-8}$ M was not statistically significant while the increases at $1·50 \times 10^{-7}$ and $3·0 \times 10^{-7}$ M were ($P < 0·05$ and $< 0·01$ respectively).

Physostigmine ($3·75 \times 10^{-8}$–$3·0 \times 10^{-7}$ M) significantly enhanced the contractions induced by ACh (Fig. 2). Physostigmine did not appreciably influence the

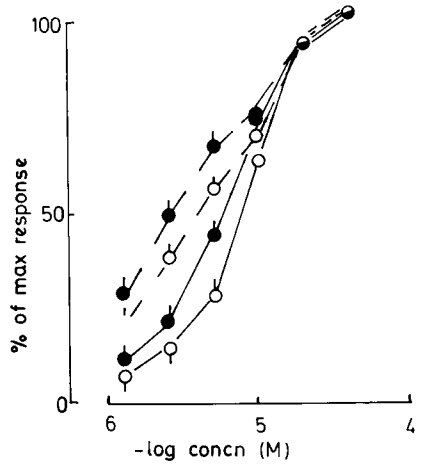

FIG. 1. Potentiation of noradrenaline-induced contractions of the rat anococcygeus muscle by physostigmine. Contractile responses were obtained to increasing concentrations of noradrenaline alone (○, ——), and in the presence of physostigmine $7·50 \times 10^{-8}$ M (○, ——) $1·50 \times 10^{-7}$ M (○, – – –) and $3·00 \times 10^{-7}$ M (○, – – –). Each point is the mean ± s.e. of, at least, 5 observations. Vertical axis represents response expressed as percent of maximal and horizontal axis represents the negative log (molar) concentrations of noradrenaline.

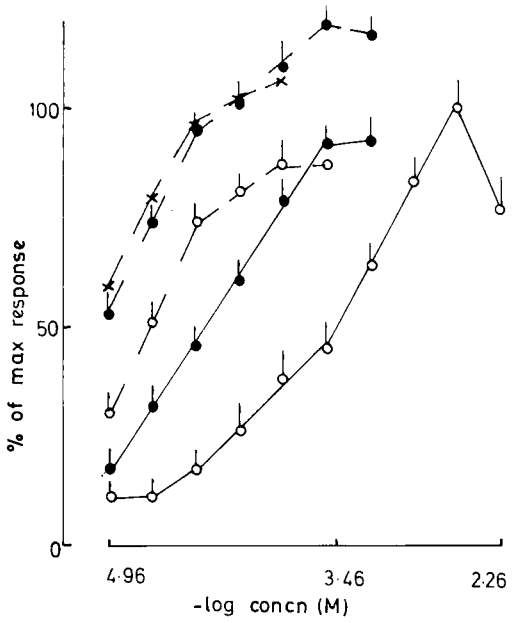

FIG. 2. Effect of various concentrations of physostigmine on ACh-induced contractions of the rat anococcygeus muscle. Responses to ACh (○, ——) were potentiated by physostigmine $3{\cdot}75 \times 10^{-8}$ (○, ——); $7{\cdot}50 \times 10^{-8}$ (○, – – –); $1{\cdot}50 \times 10^{-7}$ (○, – – –) and $3{\cdot}00 \times 10^{-7}$ M (X, – – –). Each point is the mean ± s.e. of 5 observations. Vertical axis represents response expressed as per cent of maximal and horizontal axis the negative log (molar) concentrations of ACh.

maximal except at $1{\cdot}5 \times 10^{-7}$ M, however, the −log EC50 of ACh was increased. Thus, the control −log EC50 of 3·40 ± 0·10 was significantly increased to 4·36 ± 0·06; 4·72 ± 0·10; 4·84 ± 0·04 and 4·90 ± 0·06 by physostigmine $3{\cdot}75 \times 10^{-8}$, $7{\cdot}50 \times 10^{-8}$, $1{\cdot}50 \times 10^{-7}$ and $3{\cdot}0 \times 10^{-7}$ M respectively. In each case $P < 0{\cdot}01$.

Cocaine ($5{\cdot}20 \times 10^{-7}$–$2{\cdot}08 \times 10^{-6}$ M) produced concentration-dependent enhancement of the effects of ACh and noradrenaline. The curves were shifted to the left but the maximum was not enhanced (Fig. 3).

The effect of cocaine on ACh-induced contractions appeared to be maximal at $5{\cdot}2 \times 10^{-7}$ M cocaine because higher concentrations did not produce changes that were quantitatively greater statistically. The increase in −log EC50 of ACh in the presence of cocaine $5{\cdot}2 \times 10^{-7}$M was just significant ($P < 0{\cdot}05$).

To elucidate the mechanism of potentiation further, carbachol and methoxamine were used. Carbachol is not a substrate for cholinesterases therefore if physostigmine and cocaine were acting postjunctionally they would be expected to potentiate the effect of carbachol. Methoxamine is not a substrate for neuronal uptake (Burgen & Iversen 1965) and as such it is a useful tool to differentiate between pre- and postjunctional sites of actions of drugs (Trendelenburg et al 1970).

Carbachol ($8{\cdot}86 \times 10^{-7}$–$2{\cdot}84 \times 10^{-6}$ M) and methoxamine ($8{\cdot}8 \times 10^{-7}$ M–$5{\cdot}6 \times 10^{-5}$ M) produced concentration-dependent contractions of the muscle. The −log EC50 values were 5·34 ± 0·11 (n = 4) and 5·62 ± 0·05 (n = 4) for carbachol and methoxamine respectively. Neither cocaine ($5{\cdot}2 \times 10^{-7}$–$2{\cdot}08 \times 10^{-6}$ M) nor physostigmine ($3{\cdot}75 \times 10^{-8}$ M–$3{\cdot}0 \times 10^{-7}$ M) produced any significant alteration in the effect of the agonists.

Discussion

It is undoubtedly established that physostigmine and other anticholinesterases potentiate the effects of ACh by inhibiting cholinesterases that destroy ACh in cholinergically innervated tissues (Brimblecombe 1974), and in some non-nervous tissues with cholinergic systems (Sastry & Sadavongvivad 1979). In the present study, physostigmine potentiated both ACh and noradrenaline. Potentiation of noradrenaline-induced contractions by physostigmine was not expected but anticholinesterase drugs have been found to potentiate the responses of the guinea-pig isolated vas deferens to pre- and postganglionic stimulation (Birmingham 1966), the latter probably being a potentiation of endogenously released noradrenaline. There are at least two possible explanations of the potentiation by physostigmine of noradrenaline-induced contractions: (i) physostigmine could act prejunctionally to influence noradrenergic mechanisms, or (ii) act postjunctionally to sensitize α-noradrenoceptors. The second suggestion seems negated because responses to carbachol and methoxamine were not similarly affected. A postjunctional potentiation would

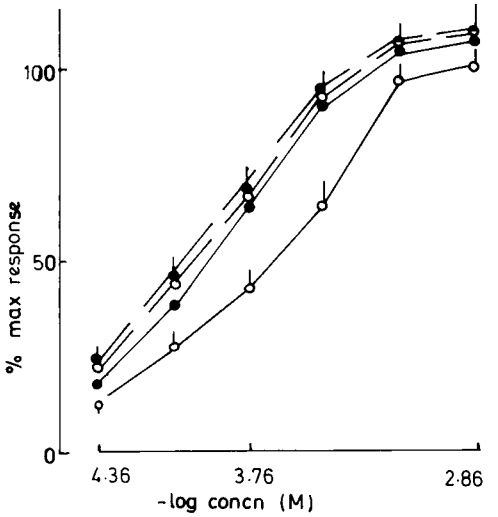

FIG. 3. Potentiation of ACh-induced contractions by cocaine. Responses to ACh (○, ——) were potentiated by cocaine, $5{\cdot}20 \times 10^{-7}$ (●, ——); $1{\cdot}04 \times 10^{-6}$ (○, – – –) and $2{\cdot}08 \times 10^{-6}$ M (●, – – –). There was no significant difference in the effect of one concentration of cocaine and another ($P > 0{\cdot}05$). Vertical axis denotes the per cent of maximal response and horizontal axis the negative log (molar) concentrations of ACh. Each point is the mean ± s.e. of, at least, 4 observations.

have been non-specific. Physostigmine acts prejunctionally to modify the effect of noradrenaline: this could be by neuronal uptake inhibition. To test this, methoxamine, a directly acting α-noradrenoceptor agonist resistant to monoamine oxidase and catechol-*O*-methyl-transferase (Bowman & Rand 1980) was used. The drug also has a low affinity for neuronal uptake (Burgen & Iversen 1965). Physostigmine failed to influence the responses of the muscle to methoxamine indicating that supersensitivity to noradrenaline in the presence of physostigmine cannot be due to an interaction postjunctionally. It therefore appears probable that the effect of physostigmine seen is prejunctional in origin.

Cocaine potentiates the effects of noradrenaline and ACh. Potentiation of noradrenaline-induced effects by cocaine is generally considered to be the result of uptake inhibition (See Trendelenburg 1973). On the other hand, there are many examples of potentiation which cannot be explained satisfactorily on the basis of neuronal uptake inhibition (Bevan & Verity 1967; Katsuya & Goto 1968; Nakatsu & Reiffenstein 1968; Reiffenstein 1968; Kalsner & Nickerson 1969; Varma & McCullough 1969; Davidson & Innes 1970; Maxwell & Eckhardt 1973; Reiffenstein & Triggle 1974). In these cases, it has been proposed that cocaine acts on α-noradrenoceptors to potentiate noradrenaline. Carpenter & Faunch (1976) obtained a potentiation of noradrenaline by cocaine in the presence of phenoxybenzamine in the rat anococcygeus muscle and concluded the effect to be prejunctional.

The present results also appear to suggest a prejunctional locus of action because neither the effect of methoxamine nor that of carbachol was influenced by cocaine. Potentiation by cocaine of ACh-induced contraction was unexpected. An interaction may exist between noradrenergic and cholinergic systems of the rat anococcygeus (Tayo 1982) since atropine reduced the effect of noradrenaline despite the insignificant contribution of cholinergic neurons (less than 5%) in this muscle (Gillespie 1980). The most attractive speculation is the probability that cocaine might have an anticholinesterase action. Westfall et al (1974) reported that when vasa deferentia are removed from rats pretreated with the anticholinesterase, disulfoton, log dose-response curves to ACh have greater maxima and lie to the left of the curves determined in tissues from untreated rats. Pennefather (1976) obtained an enhancement by cocaine of the reactivity of the vas deferens to ACh but not to carbachol.

In conclusion, it is suggested that physostigmine might inhibit neuronal uptake of noradrenaline, and cocaine inhibit acetylcholinesterases, in the anococcygeus muscle in view of its specialized architecture (Nash et al 1974).

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