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Does cocaine have a post-synaptic action on rat anococcygeus muscle?

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Cocaine is generally considered to potentiate responses to noradrenaline by inhibiting neuronal uptake of the amine. There are, however, numerous examples of potentiation which cannot be wholly explained on this basis (Bevan & Verity, 1967; Reiffenstein, 1968; Kalsner & Nickerson 1969; Maxwell & Eckhardt, 1973; Reiffenstein & Triggle, 1974). In such cases, it has been proposed that cocaine potentiates by acting on the α-adrenoceptors mediating the response (Nakatsu & Reiffenstein, 1968). These authors, using rat vasa deferentia, showed that cocaine caused an increase in the maximum response of tissues which had been treated with an irreversible antagonist (phenoxybenzamine), at a dose sufficient to reduce the maximum response to noradrenaline. As they used equilibrium responses to supramaximal doses of noradrenaline, they concluded that as the proportion of receptors not alkylated by phenoxybenzamine was insufficient to allow a maximum response, the increase in response to noradrenaline caused by cocaine could not be due to a local increase in noradrenaline concentration.

Trendelenburg (1973) has suggested that uptake inhibition is likely to be the mechanism by which cocaine potentiates responses to noradrenaline in tissues with rich noradrenergic innervations, whereas, if potentiation is seen in tissues with sparse noradrenergic innervations, then it is likely to be due to a post-synaptic action.

To test this, we examined the effect of cocaine on responses of isolated rat anococcygeus muscles to supramaximal doses of noradrenaline, both before and after treatment with phenoxybenzamine. This tissue was selected because of its dense noradrenergic innervation.

In each preparation, a log concentration-effect curve to noradrenaline was determined using equilibrium responses, after which the tissue was exposed to phenoxybenzamine at 10^{-8}M or 10^{-7}M for 5 min. The tissue was then washed periodically for 30 min after which responses to an approximate ED50 of noradrenaline were obtained at 5 min intervals until constant responses were obtained (this was never less than 60 min after phenoxybenzamine). Log concentration-effect curves to noradrenaline were then obtained in the absence and presence of cocaine at 4×10^{-6} and 2×10^{-5}M (Fig. 1).

Cocaine shifted the response curves after phenoxybenzamine to the left, indicating either a change in affinity or an increase in the local concentration of noradrenaline. When maximum responses after phenoxybenzamine in the presence of cocaine were compared with the maximum responses after phenoxybenzamine

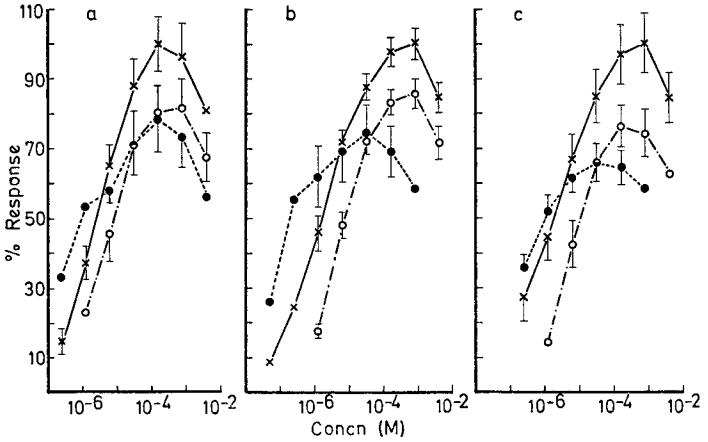

FIG. 1. Effect of cocaine on response of rat anococcygeus muscle to noradrenaline. Mean responses ± s.e.m. expressed as percentages of mean maximal response in each control curve. In no case was the maximum response after phenoxybenzamine in the presence of cocaine significantly greater than the maximal response after phenoxybenzamine in the absence of cocaine. n = 8. S.e.m. bars are omitted when n ≠ 8 (n = 2 to 7). ×——× control, ○—·—·—○ after phenoxybenzamine, ●– – – – ● after phenoxybenzamine in the presence of cocaine. The drug treatments were as follows: Phenoxybenzamine (a) at 10^{-8} M for 5 min, cocaine at 4×10^{-6} M; (b) at 10^{-8} M for 5 min, cocaine at 2×10^{-5} M; (c) at 10^{-7} M for 5 min, cocaine at 2×10^{-5} M.

* Correspondence.

only, using the Wilcoxson matched-pairs signed-ranks test, there was no significant difference for maxima after phenoxybenzamine at 10^{-8}M and maxima after phenoxybenzamine in the presence of cocaine at 4×10^{-6}M.

In the other two cases, the maxima were significantly smaller ($0{\cdot}01 > P > 0{\cdot}005$ and $P = 0{\cdot}01$ respectively). This indicates that, in this preparation, cocaine does not increase the apparent efficacy or intrinsic activity of the noradrenaline α-adrenoceptor interaction. In view of the specialized architecture of the rat anococcygeus muscle (Nash, Gillespie & Robertson, 1974), we would suggest that the leftwards shift is due to increases in the local concentration of noradrenaline, a result in keeping with Trendelenburg's prediction. The post-synaptic potentiation found by Nakatsu & Reiffenstein (1968) in the relatively densely innervated rat vas deferens should, perhaps, be considered as an exception to the general rule proposed by Trendelenberg (1973).

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Chemical aspects of penicillin allergy: mechanism of imidazole-catalysed penicilloylation

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The principal antigenic determinant in penicillin allergy is the penicilloyl group, which is thought to be formed by reactions of penicillin with nucleophilic groups of protein such as ε-amino-groups of lysine residues (for reviews, see Schwartz, 1969; Schneider, 1970). Since penicilloylation of primary amino-groups at physiological pH and temperature only proceeds slowly (Tsuji, Yamana & others, 1975; Bundgaard, 1975), catalysis is likely to be important in the immunochemical binding of penicillins to serum proteins or other tissue macromolecules. Aminolysis of penicillins by imidazole is an efficient process at neutral pH (Bundgaard, 1971, 1972a, b) and imidazole-catalysed penicilloylation could be a pathway involved in the formation of penicilloyl-protein conjugates *in vivo* (Bundgaard, 1972c; Yamana, Tsuji & others, 1975). This possibility is supported by studies involving the blocking of imidazole-groups in proteins (Wagner, Truex & Hall, 1973).

The imidazole-catalysed penicilloylation of amino- or hydroxyl-groups has been suggested to proceed either *via* the initially formed product in the reaction of penicillin with imidazole, *N*-penicilloylimidazole, or *via* its intramolecularly isomerized product, penicillenic acid (Bundgaard, 1972a). Since penicillins which are structurally incapable of undergoing rearrangement into penicillenic acids have been demonstrated to be as immunogenic as e.g. benzylpenicillin (Schneider & de Weck, 1966; Schneider, 1970), it is important to know whether the highly reactive *N*-penicilloylimidazole formed from all types of penicillins is able to transfer its penicilloyl group to various functional groups of proteins with the formation of more stable penicilloyl compounds. Experiments with 6-ethoxycarbonylaminopenicillanic acid (ethoxypenicillin) have led to the conclusion that *N*-penicilloylimidazole is capable of penicilloylating amino- and hydroxy-groups in intermolecular reactions (Bundgaard, 1972c). This conclusion has recently been contested by Yamana & others (1975) for the following reasons: (1) ethoxypenicillin itself as well as *N*-ethoxypenicilloylimidazole are not, as concluded by Bundgaard (1972c), unable to rearrange into ethoxypenicillenic acid; (2) 6-(α-toluensulphonamido)-penicillanic acid which cannot form a penicillenic acid, did not give rise to any significant penicilloylamide formation through reaction with imidazole in the presence of ε-aminocaproic acid, conditions under which benzylpenicillin as well as ethoxypenicillin readily reacted to produce penicilloylated ε-aminocaproic acid. Therefore the authors concluded that the intermolecular imidazole-catalysed penicilloylation of amino-groups by benzylpenicillin does not proceed through the intermediary *N*-benzylpenicilloylimidazole, but exclusively through its isomerized product, benzylpenicillenic acid.

I now wish to present results that support the original statement of the reactivity of *N*-penicilloylimidazole (Bundgaard, 1972c) and that also differ from the conclusions drawn by Yamana & others (1975) about the mechanism of the intermolecular