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Effect of cocaine on the affinity of α-adrenoceptors for noradrenaline

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Summary

1. Doses of cocaine which cause specific or unspecific supersensitivity in cat spleen did not alter the blocking effect of phenoxybenzamine on the responses of isolated cat spleen strips to noradrenaline.

2. The same doses of cocaine did not increase the protection of α-adrenoceptors given by noradrenaline during a standard exposure to phenoxybenzamine.

3. It is concluded that cocaine does not change the affinity of the α-adrenoceptor for noradrenaline, and therefore changes in affinity are not responsible for the potentiating action of cocaine.

Introduction

Many workers (Varma & McCullough, 1969; Kalsner & Nickerson, 1969; Davidson & Innes, 1970; Innes & Karr, 1971) have cast doubt on the uptake hypothesis (Macmillan, 1959; Furchgott, Kirpekar, Rieker & Schwab, 1963; Trendelenburg, 1966) in explaining the supersensitivity to sympathomimetic amines induced by cocaine. Their results suggest that a postsynaptic action of cocaine might be at least partly responsible for the supersensitivity.

In 1937 Clark put forward the hypothesis that cocaine might increase the rate of association of an agonist to its receptor or decrease the rate of dissociation, thus altering the affinity of the receptor for the agonist. Maxwell, Plummer, Povalski, Schneider & Coombs (1959) suggested that cocaine might produce its action by a change in the configuration of the receptor, i.e. deformation of the receptors. This hypothesis was challenged by Green & Fleming (1968), who reasoned that if a sensitizing procedure deforms the receptors it must result in a change of affinity of noradrenaline for the α-adrenoceptor, a change which should be detectable by determination of the pA_x values of a reversible antagonist like phentolamine. Green & Fleming (1968) and Davidson (1970) found that cocaine did not alter the pA_x values in isolated strips of cat spleen. However, pA_x does not vary with the affinity of the agonist but of the antagonist. Therefore the affinity of noradrenaline might be substantially altered without a change in affinity of the receptor for phentolamine.

Innes & Karr (1971) showed that adrenaline prevented the usual development of supersensitivity in isolated spleen strip exposed to cocaine. It was suggested that adrenaline prevented cocaine from combining with the site of its sensitizing action. If combination between adrenaline and the α-adrenoceptor were responsible for

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blocking the action of cocaine, it is possible that cocaine may act at an allosteric site related to the α-adrenoceptor. Combination of cocaine at such a site might well deform the α-adrenoceptor and thereby alter its affinity for catecholamines.

We have therefore tested the affinity of the α-adrenoceptors for noradrenaline by determining the ability of noradrenaline to protect against block by phenoxybenzamine, a non-equilibrium α-adrenoceptor blocker. Since the degree of protection should depend on the affinity of the α-adrenoceptors for noradrenaline, the results should indicate whether cocaine modifies the receptors. To circumvent the possibility that cocaine may alter the affinity for the antagonist also, we first had to establish that cocaine did not alter the blocking effect of phenoxybenzamine.

Methods

Cats (0·6–1·2 kg) of either sex were killed by a blow on the head. The spleen was removed and placed in Krebs–Henseleit solution at 4° C. Strips 20 mm long and 3 mm wide were cut from the edge of the spleen and placed in individual 10 ml organ baths containing Krebs–Henseleit solution of the following composition (mM): NaCl, 118 ; KCl, 4·7 ; $CaCl_2$, 2·5 ; KH_2PO_4, 1·1 ; $MgSO_4$, 1·2 ; $NaHCO_3$, 25·0 ; glucose, 11·0. The solution was kept at 37° C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Strips were allowed to equilibrate for 1 h before the experiment was started. Isotonic contractions against 1 g tension were recorded on a kymograph at 6 times magnification.

Block of α-adrenoceptors by phenoxybenzamine in the presence and absence of cocaine

After a full cumulative dose-response curve to noradrenaline was determined on three strips from the same spleen, the preparations were washed every 10–15 min until they completely relaxed. Two strips were then treated with cocaine (1 μg or 30 μg/ml). Five minutes later, without washing, one cocaine-treated strip and one untreated strip were exposed to phenoxybenzamine (0·1 μg/ml) for a further 5 minutes. All drugs were then washed out and the bathing fluid was changed twice. Subsequently the strips were washed every 10–15 min for a period of 90 or 150 min depending on whether the smaller (1 μg/ml) or larger (30 μg/ml) dose of cocaine had been used. At the end of this period, the dose-response curve to noradrenaline was determined again.

Since the cat spleen has no spare α receptors (Davidson & Innes, 1972) the maximum responses before and after application of phenoxybenzamine both represent occupation of all available receptors. The maximum response after phenoxybenzamine expressed as a percentage of the maximum response before phenoxybenzamine should therefore give an approximate measure of the proportion of receptors which have been blocked by phenoxybenzamine. Changes in this value due to cocaine should reflect changes in the affinity of the receptors for phenoxybenzamine.

Protection experiments

The method used was similar to that used by Davidson (1970) to compare the affinities of sympathomimetic amines for the α-adrenoceptor. A cumulative dose-response curve to noradrenaline was determined on four strips from the same cat.

After relaxation, two strips were treated with cocaine, 1 μg and 30 μg/ml respectively. Five min later, these two strips and one untreated strip were exposed to noradrenaline, 100 μg/ml, for 5 minutes. No noradrenaline was added to the fourth strip, which was used as an unprotected control strip. All four strips were then exposed to phenoxybenzamine, 0·1 μg/ml, for 5 min, while the other drugs remained in the bath. After 150 min, during which the strips were washed frequently, the dose-response curve to noradrenaline was determined again.

The dose of noradrenaline required to cause a maximal contraction in the untreated spleen strip was 30 μg/ml. The dose selected for protection was 100 μg/ml, because it gave substantial protection in preliminary experiments and did not cause desensitization which would interfere with subsequent responses. A dose of 1 mg/ml gave much greater protection, but left considerably less possibility of improved protection by cocaine, and also led to problems of desensitization.

The drugs used were (−)-noradrenaline bitartrate monohydrate (Calbiochem), cocaine hydrochloride (British Drug Houses), phenoxybenzamine hydrochloride (Dibenzyline ; Smith, Kline & French). Drug concentrations refer to the final bath concentration expressed as free base for noradrenaline and as salt for cocaine and phenoxybenzamine.

The data were analysed by the *t* test for paired data (Goldstein, 1964).

Results

Effect of cocaine on block of α-adrenoceptors by phenoxybenzamine

Thirteen experiments were done to test the effect of cocaine 1 μg/ml and 30 μg/ml on the α-adrenoceptor block produced by phenoxybenzamine (0·1 μg/ml). The contraction due to a maximal dose of noradrenaline after phenoxybenzamine was expressed as a percentage of the maximum response before phenoxybenzamine. In six experiments the maximal responses to noradrenaline were reduced to $36{\cdot}6 \pm 5{\cdot}1\%$ without cocaine and $34{\cdot}7 \pm 5{\cdot}1\%$ in the presence of cocaine, 1 μg/ml. With the larger dose of cocaine, 30 μg/ml, the corresponding results of 7 experiments were $44{\cdot}3 \pm 4{\cdot}4\%$ without cocaine and $39{\cdot}7 \pm 6{\cdot}7\%$ with cocaine. Neither concentration of cocaine significantly altered the intensity of block by phenoxybenzamine.

In order to ensure that the block by phenoxybenzamine was assessed after potentiation by cocaine had disappeared, responses to noradrenaline were tested in a third strip which was not treated by phenoxybenzamine. The strip was exposed to cocaine (1 or 30 μg/ml) for 10 min as in the strips treated with phenoxybenzamine and tested 90 or 150 min later. At this time the dose-response curve to noradrenaline was not shifted to the left and therefore responses to noradrenaline were not potentiated. In addition, strips treated with cocaine and phenoxybenzamine were exposed a second time to cocaine after the maximum response after phenoxybenzamine had been determined. The second dose of cocaine increased the responses to noradrenaline.

Effect of cocaine on the protection given by noradrenaline against block by phenoxybenzamine

Noradrenaline, 100 μg/ml, was used in 8 experiments to protect the α-adrenoceptors against block by phenoxybenzamine, either alone or in the presence of

cocaine 1 μg/ml or 30 μg/ml. The maximum responses after phenoxybenzamine were $36{\cdot}5 \pm 6{\cdot}0\%$ for unprotected strips; in the strips protected by noradrenaline the maximum responses were $60{\cdot}6 \pm 7{\cdot}0\%$ without cocaine, $57{\cdot}0 \pm 5{\cdot}2\%$ with cocaine 1 μg/ml and $56{\cdot}8 \pm 6{\cdot}5\%$ with cocaine 30 μg/ml. The responses in unprotected strips were significantly less than in all three groups of protected strips. The results in the cocaine-treated strips were not significantly different from those without cocaine.

Discussion

Our results do not support the hypothesis that cocaine increases the affinity of the α-adrenoceptor for noradrenaline. Cocaine failed to alter the degree of block caused by phenoxybenzamine and therefore the affinity of the receptor for phenoxybenzamine. The protecting action of noradrenaline against block by phenoxybenzamine was not increased by either of the concentrations of cocaine tested. An increase in affinity of the receptor for noradrenaline should have afforded better protection.

In estimating the degree of block by phenoxybenzamine due to exposure in the presence of cocaine, the possibility was considered that the subsequent responses to noradrenaline were still enhanced by cocaine. However, the experiment was designed to account for this possibility; strips were given a standard exposure to phenoxybenzamine at the end of which the antagonist was washed out, and the cocaine was washed out at the same time. The subsequent response to noradrenaline was tested only when the responses of control strips treated with cocaine but not phenoxybenzamine had shown the potentiating action of cocaine had worn off. This still left the possibility that phenoxybenzamine might prolong the action of cocaine, but this possibility was discounted by the experiments which showed that cocaine again increased the responses to noradrenaline which had been found in the test after phenoxybenzamine. These results agree with the finding of Lewis & Miller (1966) and Green & Fleming (1967, 1968) that cocaine does not affect block by phenoxybenzamine. Some support is also provided by the conclusion of Lewis & Miller (1966) that cocaine does not change the amount of ^{3}H-phenoxybenzamine taken up by seminal vesicles of the rat. However, the uptake of labelled phenoxybenzamine by a tissue does not provide convincing evidence of uptake by α-adrenoceptors, because phenoxybenzamine is known to bind to many tissue components.

The basis of the protection experiments is that an increase in affinity should increase the protection by noradrenaline. However, the possibility must be considered that the degree of protection provided by noradrenaline in the strips without cocaine was already maximum and therefore no further change could be detected. This, however, is not the case, since Davidson (1970) showed that the same concentration of adrenaline, 100 μg/ml, which has a greater affinity than noradrenaline for the α-adrenoceptor in spleen strips, gives greater protection than does noradrenaline. The protection method should therefore be adequate to detect increases in affinity for noradrenaline.

The doses of cocaine tested, 1 μg/ml and 30 μg/ml, were selected because these concentrations cause qualitatively different types of supersensitivity. Davidson (1970) reported that 1 μg/ml caused supersensitivity of cat spleen strips specific for catecholamines whereas the larger dose, 30 μg/ml, increased the supersensitivity to catecholamines, but also induced supersensitivity to histamine, acetylcholine and

angiotensin. It appears from our results, therefore, that the supersensitivity to catecholamines is not due to changes in affinity of the α-adrenoceptor with doses of cocaine which cause either specific or unspecific supersensitivity.

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