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Protection against induction of supersensitivity to catecholamines by cocaine

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Summary

1. Adrenaline, noradrenaline, isoprenaline, tyramine, phentolamine, pronethalol, histamine and acetylcholine were each tested for their ability to prevent cocaine from causing supersensitivity to catecholamines in cat spleen strips *in vitro*. A high concentration of one of these drugs was added to the bath 5 min before cocaine hydrochloride (10 μg/ml). The effect on subsequent responses to catecholamines was compared with the effect of cocaine in control strips in the absence of an interfering drug.

2. Phentolamine completely abolished the potentiating effect of cocaine. Large doses of adrenaline or noradrenaline reduced, but did not completely prevent, potentiation. Tyramine, isoprenaline, pronethalol, histamine and acetylcholine did not prevent potentiation.

3. The ability of these drugs to interfere with potentiation does not correlate well with their ability to interfere with uptake of noradrenaline. Interference with uptake by cocaine is therefore unlikely to account fully for potentiation.

Introduction

Cocaine causes in a variety of tissues, supersensitivity which is relatively specific for catecholamines (Innes & Kosterlitz, 1954; Trendelenburg, 1963). The mechanism of this action remains unknown, although the demonstrated ability of cocaine to inhibit tissue uptake of catecholamines (Whitby, Hertting & Axelrod, 1960; Hertting, Axelrod & Patrick, 1961; Dengler, Spiegel & Titus, 1961; Hertting, Axelrod & Patrick, 1962) is currently offered as an explanation (Macmillan, 1959; Trendelenburg, 1963, 1966; Furchgott, Kirkpekar, Rieker & Schway, 1963; Axelrod, 1965). Clark (1937) suggested that supersensitivity may be due to altered affinity of the receptors for agonists, and Maxwell, Sylwestrowicz, Holland, Schneider & Daniel (1961) repeated this suggestion to account for supersensitivity due to methylphenidate. The possibility that cocaine may potentiate catecholamines by changing this property of the effector cells has never been confirmed or rejected.

Several investigators dealing with enzyme systems have provided evidence that allosteric interactions may occur with many activators and inhibitors, wherein the conformation of the receptor site, and hence its affinity for substrate, is influenced by the attachment of other molecules at specific sites remote from the receptor (Koshland, 1963, 1964; Monod, Changeux & Jacob, 1963). Similarly, cellular

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receptor systems might undergo analogous conformational changes in affinity under the influence of allosteric activators and inhibitors, which could thus alter the sensitivity of the cell to agonists. Combination of cocaine with cellular sites so as to affect the adrenoceptive receptor site could accordingly be responsible for the observed supersensitivity. If cocaine has this effect it should be possible to prevent the potentiating action by preventing the combination of cocaine at the receptor site. We have studied the effects on the development of supersensitivity due to cocaine of the simultaneous presence of various drugs which act on smooth muscle and might therefore compete with cocaine for its site of action.

The concept of competition for a common site has been applied previously to identify the specificity of drug-receptor interactions. Thus Furchgott (1954) demonstrated different receptors for acetylcholine, histamine, adrenaline and 5-hydroxytryptamine in rabbit aorta. All these agonists contracted this tissue, and dibenamine blocked the response in each case. However, if a large dose of any one of the agonists was added before the dibenamine, subsequent responses to the usual doses of this agonist were not blocked, while responses to the other agonists were prevented. This was interpreted as indicating that the large concentration of agonist combined only with its own specific receptors, thus selectively protecting them from the antagonist while receptors for other types of agonist were blocked. Innes (1962) used this technique to show that in the cat spleen adrenaline and 5-hydroxytryptamine provided mutual protection against block of responses to either agent by phenoxybenzamine. This was taken as an indication that in this organ both agents cause a response by activating the same receptors. On the same principle, it should be possible to establish the importance of competitive interaction with a common site to the supersensitivity caused by cocaine; adequate concentrations of other substances with sufficient affinity for the same site should protect the tissue from becoming sensitized when cocaine is present.

Methods

Cats (0·7–2·5 kg) of either sex were killed by a blow on the head, and the spleens were immediately removed into Krebs-Henseleit solution at 4° C. For each experiment four strips, each 20 mm long, 4–5 mm wide, and about 2 mm thick, were cut from the edge of a single freshly removed spleen. Each strip was anchored with a loop of fine thread to a glass hook inside a 10 ml tissue bath and the free end was attached by a thread to a light Palmer frontal writing lever. An equilibration period of 1 h was then allowed before drugs were added. During this time, and throughout the experiment when drugs were not being added to or removed from the bath, the bathing fluid was routinely changed at 10–15 min intervals. Isotonic contractions against 1 g tension were recorded on a kymograph at 8 times magnification. The tissues were bathed in Krebs-Henseleit solution of the following composition: NaCl, 118 mM; KCl, 4·7 mM; $CaCl_2$, 2·4 mM; KH_2PO_4, 1·1 mM; $MgSO_4$, 1·2 mM; $NaHCO_3$, 25 mM; glucose, 11 mM. The solution was continuously bubbled with 5% CO_2 in oxygen before and during the experiment, and the temperature kept at 38° ±0·5° C.

Drugs

(−)-Noradrenaline bitartrate monohydrate, (−)-adrenaline bitartrate, (±)-isoprenaline HCl (Winthrop), tyramine HCl, histamine acid phosphate, acetylcholine Cl,

cocaine HCl, and phentolamine HCl (Rogitine, Ciba), and pronethalol HCl (Alderlin, Ayerst) were used in these experiments. Stock solutions were prepared in distilled water, or in 0·1 N HCl for the catecholamines, tyramine and acetylcholine, and were refrigerated when not in use. For each experiment, fresh dilutions in 0·9% NaCl were prepared in a range of concentrations sufficient that additions to the bath rarely exceeded 0·5 ml. Catecholamine dilutions were acidified with HCl, 0·01 N. All concentrations are expressed as grammes of free base in 1 ml of solution.

Selection of the potentiating dose of cocaine

Cocaine hydrochloride, 10 ng–10 μg/ml, potentiates catecholamines in the cat spleen strip. We wished to test the effect of protecting doses of various agonists and antagonists against the lowest possible concentration of cocaine, which would thus favour the protecting drug in its competition for common sites of action. However, some of the protecting drugs had other actions, stimulant, potentiating or inhibitory, which did not disappear immediately after their removal from the bath.

Tests of potentiation therefore had to be delayed till these effects of the potentiating drugs had disappeared in the control strips. The potentiating effect of the smaller doses of cocaine hydrochloride, 10 ng–1 μg/ml, also disappeared during this period. We therefore had to use a higher concentration of cocaine hydrochloride, 10 μg/ml, whose potentiating effect outlasted the effects of most of the protecting drugs long enough to allow subsequent tests of potentiation.

Protocol

The experimental design is shown in Table 1. The testing agonist was noradrenaline in some experiments, but was usually adrenaline. The choice of agonist did not appear to be important, since adrenaline and noradrenaline were equally potentiated by cocaine and several duplicate experiments showed that the results were similar whether adrenaline or noradrenaline was used as the test agonist. Reproducible control responses to noradrenaline or adrenaline (0·1–3 μg/ml) were obtained in each of four strips from the same spleen. The drug being tested for its effect on cocaine potentiation was then added in a high concentration to two of the strips. Five minutes later, cocaine hydrochloride, 10 μg/ml, was added to one of these strips and to one other strip. After another 5 min all drugs were removed, and the tissues were washed at 5 min intervals for 30 minutes. Responses to the catecholamine were then tested regularly until the response of the tissue which received only protecting drug returned to its original value. The responses in each

TABLE 1. *Design for experiments on protection against cocaine-induced supersensitivity*

Procedure	Strip			
	1	2	3	4
1. Test response	X	X	X	X
2. Add protecting drug, wait 5 min	—	—	P	P
3. Add cocaine, wait 5 min, remove drugs	—	C	C	—
4. Test response	X	X	X	X

Four strips from the same spleen are used in each experiment. X denotes addition of a small dose of noradrenaline or adrenaline. P and C denote addition of protecting drug and cocaine respectively. Strip 1: time control. Strip 2: cocaine. Strip 3: protecting drug and cocaine. Strip 4: control for effect of protecting drug.

of the four strips at this time were expressed as a percentage of the control value for the same strip. Statistical significance of differences between treatments was calculated with Student's *t* test.

Results

Adrenaline, noradrenaline, tyramine, phentolamine, isoprenaline, histamine and acetylcholine were each tested for their ability to prevent potentiation of catecholamine responses by cocaine. These drugs were chosen because their known effects on smooth muscle involve combination with a variety of receptor sites. Their effects on cocaine potentiation are summarized in Table 2. The potentiation by cocaine was significant in all experiments without protection. Except for adrenaline, noradrenaline and phentolamine, the agents tested for protection failed to alter the potentiation caused by cocaine. Phentolamine completely abolished the potentiation ; adrenaline decreased the potentiation considerably, but a significant increase in the response to catecholamine remained ; the apparent reduction in potentiation due to noradrenaline was not statistically significant.

The large protecting doses of adrenaline or noradrenaline (50 μg/ml) presented a problem due to the prolonged contraction they caused. It was necessary to wait for over 1 h in most experiments, until the strip relaxed to the precontraction level, before test responses to catecholamine were determined. This long delay probably accounts for the smaller potentiation caused by cocaine alone in some experiments where noradrenaline was the protecting drug used. Lower concentrations of adrenaline or noradrenaline had no protecting effect.

Protection experiments with phentolamine also presented a problem due to the block of α-adrenoceptors. Tests for potentiation were delayed until phentolamine had been washed out of the strips, as indicated by return to control values of the strips receiving phentolamine alone. At this time, cocaine potentiated noradrenaline in the unprotected strips but not in the strips protected with phentolamine. These results can possibly be interpreted as a prolongation by cocaine of the blocking effect

TABLE 2. *Effect of some smooth muscle stimulants and antagonists on supersensitivity induced cocaine in isolated spleen strips*

Test drug	Protecting drug (concentration)	N	Response after cocaine hydrochloride (10 μg/ml) % of control		
			Unprotected	Protected	*P*
Adrenaline	Adrenaline (50 μg/ml)	4	234·5±20·9	149·0±14·2	<0·02
Noradrenaline	Noradrenaline (50 μg/ml)		182·6±21·6	158·6± 9·8	NS
Noradrenaline	Tyramine (50 μg/ml)	4	218·8±21·3	344·4±44·8	NS
Adrenaline	Phentolamine (3 μg/ml)	4	376·5±78·3	102·0±21·6	<0·005
Adrenaline	Isoprenaline (50 μg/ml)	4	290·8±30·4	369·7±80·5	NS
Adrenaline	Pronethalol (50 μg/ml)	4	263·5±39·6	253·0±33·7	NS
Adrenaline	Acetylcholine (50 μg/ml)	4	204·3±25·7	237·5±33·6	NS
Adrenaline	Histamine (50 μg/ml)	4	222·0±13·7	220·8±27·2	NS

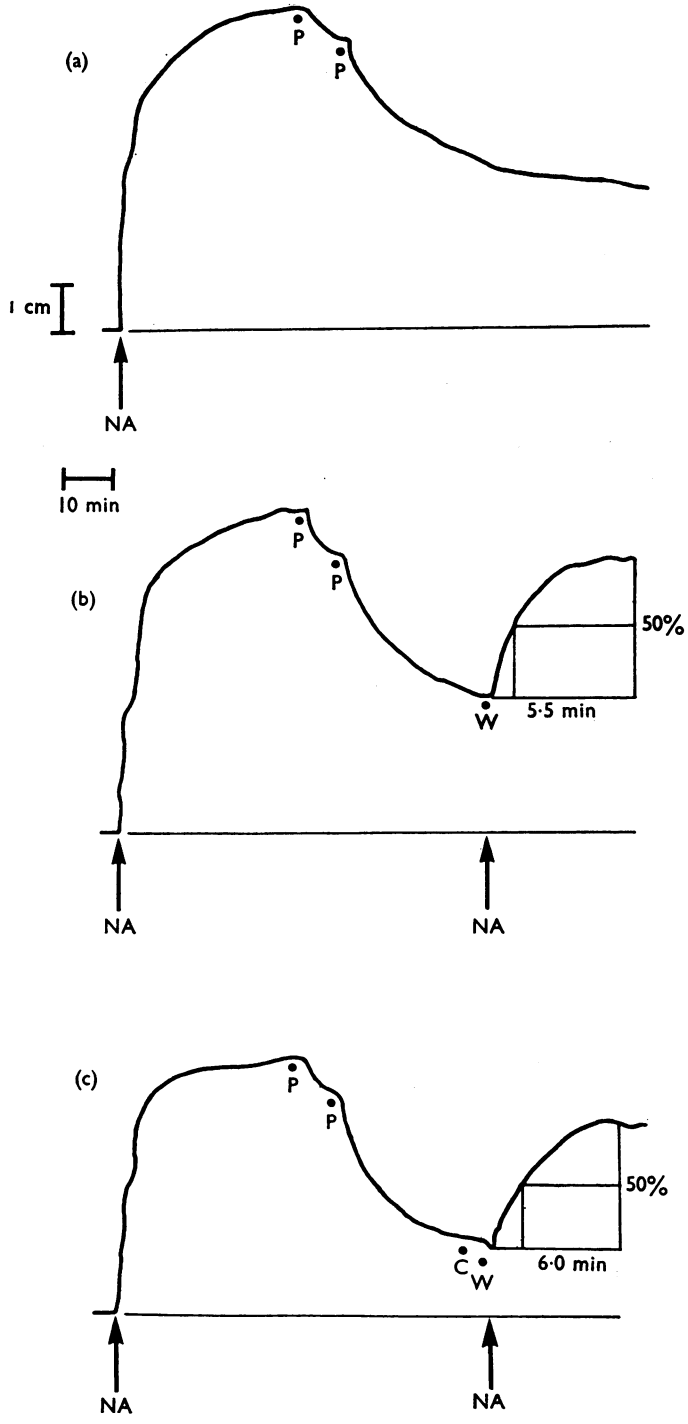

FIG. 1. Effect of cocaine on the duration of block by phentolamine. Three strips, (a), (b), (c), from the same spleen were maximally contracted by noradrenaline, 155 μg/ml (NA). At the peak of contraction phentolamine (P) was added to each strip, to a total dose of 110 μg/ml. In (c), cocaine hydrochloride, 10 μg/ml, was added at C. Five minutes later at W strips (b) and (c) were washed and noradrenaline, 155 μg/ml, was immediately added. The time to 50% of the maximum contraction was measured as shown. In a fourth strip from the same spleen, not shown, phentolamine was not added, and the maximal contraction to noradrenaline, 155 μg/ml, was sustained with little decay for the duration of the experiment.

of phentolamine, so that responses to adrenaline would return to normal sooner in the phentolamine control than in the cocaine treated strips. This possibility was tested in two experiments by examination of the effect of cocaine on the duration of phentolamine block (Fig. 1). In each experiment, four spleen strips were contracted maximally with noradrenaline (155 μg/ml). One received no further treatment and served as a control for the persistence of the contraction. The other three were given a total of 110 μg/ml of phentolamine, which reduced the contraction to a new lower level. One strip, (c), was given 10 μg/ml of cocaine hydrochloride for 5 min, after which the drugs were washed out of this and one other strip, (b). Noradrenaline (155 μg/ml) was immediately added and the contraction allowed to reach a new peak. The time to reach 50% of the subsequent contraction was taken as a measure of the recovery time (Fig. 1, (b) and (c)). The 50% rise times in the two experiments were 6·0 and 4·0 min after cocaine compared to 5·5 and 3·5 min for their respective controls. These differences are not adequate to account for the effect of phentolamine in the protection experiments.

Pronethalol given alone potentiated responses to adrenaline. This potentiation lasted approximately the same time as potentiation by cocaine. Therefore, we could not determine by this method whether pronethalol interfered with potentiation by cocaine. The data for experiments with pronethalol are also presented in Table 2. After the removal of cocaine and pronethalol from the baths, the responses to adrenaline were compared 75 to 90 min later, approximately the same time as with the other protecting agents. Pronethalol did not decrease or add to the potentiating effect of cocaine.

Discussion

Our results, although they cannot rule out an effect on catecholamine uptake as the explanation for supersensitivity due to cocaine, are not readily reconciled with this interpretation. Of the protecting agents we tested, several are known to interact with the uptake mechanism in various tissues. Noradrenaline has high affinity for uptake sites in the cat spleen (Dengler, 1965) and in rat heart (Burgen & Iversen, 1965). Tyramine acts by releasing stored noradrenaline in many tissues (Burn & Rand, 1958 ; and many others) and this effect is surmountably antagonized by cocaine (Muscholl, 1961). Furchgott *et al.* (1963) showed that tyramine prevented the inhibition of noradrenaline uptake by cocaine, suggesting that all three agents interact with a common uptake site. Dengler *et al.* (1961) showed that phentolamine was a weak inhibitor of catecholamine uptake in the cat spleen. Iversen (1965) reported similar findings in the isolated rat heart. In our experiments, where relatively large doses of the protecting agents were used, noradrenaline caused only a slight reduction in potentiation by cocaine, and tyramine was without effect. Significant potentiation occurred after incubation with cocaine in the presence of either noradrenaline or tyramine as protecting agent. In contrast, phentolamine completely prevented potentiation of noradrenaline by cocaine. Since we have not simultaneously measured tissue catecholamine concentrations in these experiments, no categorical statement can be made regarding changes in uptake. However, in view of the unremarkable effects of noradrenaline and tyramine on potentiation by cocaine, it would be surprising if the very profound effect of phentolamine were due to a significant interaction with the uptake mechanism.

Sites other than the uptake sites may fall in the general category of ' sites of loss ' (Veldstra, 1956) with which the adrenergic mediator may combine without leading to a contraction, thus theoretically reducing the active concentration. β-Adrenoceptors could be considered in this category, and Bickerton (1963) has provided evidence that these exist in cat spleen. The results of our experiments with isoprenaline indicate that any activity cocaine may have on β-adrenoceptors is unrelated to its potentiation of catecholamines in the cat spleen. Pronethalol caused a striking potentiation of noradrenaline which lasted as long as the potentiation by cocaine, and it is therefore impossible to draw any conclusions regarding the effects of pronethalol on cocaine potentiation from these experiments. Further investigation is in progress to examine the potentiation by pronethalol.

An obvious feature common to the agents which afforded protection, phentolamine and adrenaline, in our experiments is their affinity for α-adrenoceptors. If cocaine combined directly with α-adrenoceptors, it would be an antagonist rather than a potentiator. However, cocaine might influence the α-adrenoceptor by combining with a different site nearby. Molecular interactions between the drug and tissue molecules could induce conformational changes in the α-adrenoceptor resulting in increased affinity for drugs which activate it. Although there is no direct evidence for this hypothesis in cellular drug-receptor systems, it represents a close analogy with current theory regarding allosteric interactions in enzyme kinetics (for example Koshland, 1963, 1964 ; Monod *et al.*, 1963). Koshland (1964) has suggested that allosteric activators and inhibitors may mutually interact, although combining with different sites, and that the presence of one may have a stabilizing influence which decreases the combination of the other with its site. By analogy, then cocaine could be an allosteric activator of α-adrenoceptors, and phentolamine, by combining with α-adrenoceptors, could have a stabilizing influence on the receptor conformation, thus preventing α-adrenoceptor activation by the cocaine. A basic factor prompting Monod *et al.* (1963) to consider allosteric interactions was the observation that many enzyme systems respond promptly and reversibly to substances which are totally unrelated to their substrates, coenzymes or products. In this context, the analogy with cocaine is consistent, since this ' activator ' bears little resemblance to other agents which modify the adrenergic mechanism. The degree of protection offered by phentolamine, adrenaline and noradrenaline coincides with the affinity of these drugs for α-adrenoceptors of cat spleen as estimated by Davidson & Innes (unpublished observation). Phentolamine has considerably greater affinity than adrenaline for these receptors and the affinity of noradrenaline is relatively low. If combination with the α-adrenoceptors is the mechanism by which protection against potentiation by cocaine takes place, equal doses of the protecting drugs would be expected to assume the same order of potency in affording protection.

Our experiments with protection by acetylcholine and histamine, which failed to modify potentiation by cocaine, indicate that these receptors are not involved in the mechanism by which potentiation of catecholamines occurs.

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