Brit. J. Pharmacol. (1958), **13**, 6.

SENSITIZATION OF THE HEART AND NICTITATING MEMBRANE OF THE CAT TO SYMPATHOMIMETIC AMINES BY ANTIHISTAMINE DRUGS

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(RECEIVED JUNE 7, 1957)

Sensitization to the cardio-accelerator action of adrenaline and noradrenaline by five antihistamines was examined on the acutely denervated heart of the cat. Antazoline (Antistin), chlorcyclizine and promethazine (Phenergan) increased the cardio-accelerator responses to both amines equally. Mepyramine (Anthisan) increased noradrenaline more than adrenaline action. Diphenhydramine (Benadryl) resembled cocaine in potentiating the responses to noradrenaline but not to adrenaline.

On the nictitating membrane mepyramine caused sensitization to the actions of adrenaline, noradrenaline and tyramine, an effect similar to that of chronic preganglionic denervation. Diphenhydramine enhanced the action of noradrenaline more than that of adrenaline and had little effect on tyramine action, giving a sensitization which bears a greater resemblance to the type caused by cocaine or chronic postganglionic denervation.

It is suggested that two distinct mechanisms are required to account for the phenomena of sensitization.

Enhancement of the action of adrenaline by antihistamine drugs has been described by Yonkman, Chess, Mathieson, and Hansen (1946) on the nictitating membrane of the cat, and by Sherrod, Loew, and Schloemer (1947) on the blood pressure of the dog. Antihistamines have also been shown to potentiate the inotropic action of adrenaline on the isolated toad heart (Kuriaki and Uchida, 1955). Lecomte and Fischer (1952) extended the observations on the nictitating membrane, finding enhancement of the action of other sympathomimetic amines bearing a phenolic hydroxyl group in the *meta* position. As the presence of a hydroxyl group in this position is characteristic of the amines whose action is augmented by cocaine, it was suggested that the potentiating action of the antihistamines was similar to that of cocaine.

Innes and Kosterlitz (1950) observed that, in cats with acutely denervated hearts, cocaine potentiated the cardio-accelerator action of noradrenaline but not of adrenaline. It was accordingly of interest to examine the effects of antihistamines upon the cardio-accelerator action of these amines.

It was shown by Innes and Kosterlitz (1954b) that ephedrine or chronic preganglionic denervation caused sensitization of the nictitating membrane to a wide range of sympathomimetic amines, while cocaine or chronic postganglionic denervation caused sensitization to some sympathomimetic amines but a reduced sensitivity to others. The present investigation therefore includes a study of the effects of two antihistamine drugs on the unsensitized, acutely denervated nictitating membrane and on membranes previously sensitized by chronic preganglionic or postganglionic denervation.

METHODS

Experimental.—For the analysis of the cardiac effects of the drugs, spinal cats were prepared by the technique described by Kosterlitz, Krayer and Matallana (1955). The heart was acutely denervated by cutting the vagi and removing the stellate ganglia and the upper part of the thoracic sympathetic chain. In experiments where the cardio-accelerator nerves were to be stimulated, the nerves were secured before removal of the stellate ganglia. In 3 cats the stellate ganglia were removed at a preliminary operation 16 to 28 days before the experiment.

The heart rate was counted from electrocardiographic recordings. A count of 30 sec. before injection of the sympathomimetic amine gave the basal heart rate. The injection was made over a 15 sec.

period and counting was continued for 90 sec. thereafter. In addition a continuous graphic recording was made by the heart rate recorder described by Innes and Kosterlitz (1954a). In this way prolongation of the action of a drug could be more readily observed.

In 3 experiments a Ritchie-BNI rectangular wave stimulator (Walter and Ritchie, 1945) was used to apply supramaximal stimuli to the cardio-accelerator nerves. Because the stimulus artefact prevented a satisfactory recording of the electrocardiogram, the heart rate increase during the 90 sec. period following stimulation was taken to be a sufficient measure of the response to stimulation of the nerves.

Care was taken to minimize the effects of temperature variation on the heart rate by ensuring that in each experiment the body temperature was kept constant within 0.5°.

In experiments where the effects of the antihistamine drugs were studied on the nictitating membrane of the cat, pentobarbitone sodium (50 mg./kg. intraperitoneally) was used as anaesthetic. The membrane was acutely denervated by preganglionic section of the cervical sympathetic trunk. Where the action on previously sensitized membranes was to be examined, the membranes were denervated preganglionically (decentralized) or postganglionically, by removal of the superior cervical ganglion (denervated) at a preliminary operation 14 to 16 days before the experiment.

In all animals the action of the adrenals was excluded, generally by removing the glands, but in a few experiments by tying the adrenal veins.

Drugs.—Suitable dilutions of adrenaline bitartrate, noradrenaline bitartrate and tyramine acid phosphate were made in Ringer-Locke solution containing ascorbic acid (0.4 mg./ml.). Injections of 0.25 to 1 ml. were made into the femoral vein, each injection taking 15 sec. The quantities refer to the free bases.

Solutions of antazoline HCl (Antistin), diphenhydramine HCl (Benadryl), chlorcyclizine HCl, mepyramine maleate (Anthisan) and promethazine HCl (Phenergan) were made in distilled water. The doses of these drugs and of cocaine HCl refer to the salts.

RESULTS

The Effect of Antihistamines on the Cardio-accelerator Action of Adrenaline and Noradrenaline.

Table I shows the potentiating effect of antihistamines on the cardio-accelerator action of adrenaline and noradrenaline on the acutely denervated heart.

TABLE I

POTENTIATION OF CARDIO-ACCELERATOR ACTION OF ADRENALINE AND NORADRENALINE BY ANTIHISTAMINES

The symbol + refers to an increase in the response to the amine. The asterisk refers to the results of Innes and Kosterlitz (1950, 1951).

Drug	Dose (mg./kg.)	No. of Experiments	Effects on Cardio-acceleration Caused by	
			Adrenaline	Noradrenaline
Diphenhydramine	5–10	3	0	+++
Mepyramine	1–5	5	+	++
Antazoline	1–3	3	+	+
Chlorcyclizine	5–10	3	+	+
Promethazine	2·5–20	4	+	+
Cocaine	2	*	0	+++

On the acutely denervated cat heart, noradrenaline normally causes little cardio-acceleration in comparison with like doses of adrenaline; after cocaine, noradrenaline action is increased to such an extent that it equals or exceeds the action of adrenaline (Innes and Kosterlitz, 1950, 1951). Of the antihistamines examined diphenhydramine alone caused a selective augmentation of noradrenaline cardio-acceleration as did cocaine (Fig. 1). Although mepyramine increased noradrenaline more than adrenaline action, a full cocaine-like effect was not obtainable, since the noradrenaline responses in no case became equal to those of adrenaline.

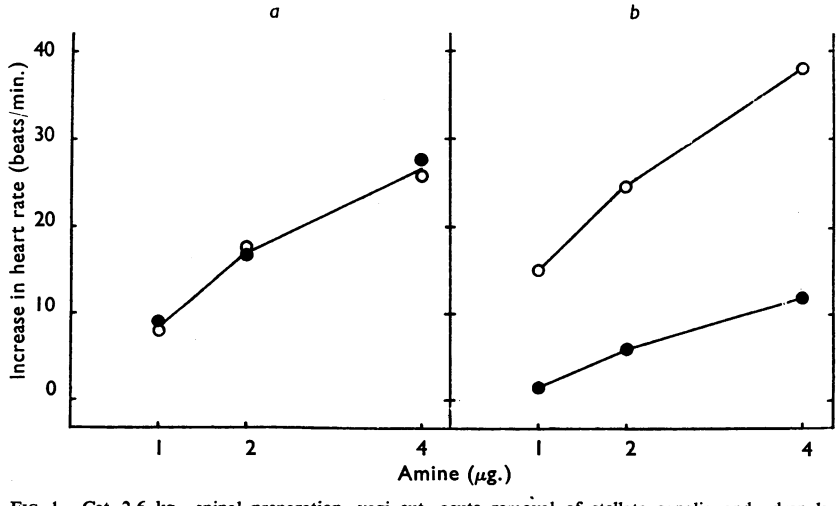

FIG. 1.—Cat, 2.6 kg., spinal preparation, vagi cut, acute removal of stellate ganglia and adrenals. Dose/response curves of increase in heart rate (beats/min.). ●——● control; O——O after diphenhydramine (10 mg./kg.). *a*, adrenaline; *b*, noradrenaline.

After the full effect of the antihistamines had been recorded, cocaine (2 mg./kg.) was injected in order to test whether a further increase in the noradrenaline responses was possible. Cocaine caused no further potentiation after diphenhydramine; with the remaining antihistamines, noradrenaline responses became equal to those of adrenaline only after the subsequent administration of cocaine.

The effect of mepyramine was examined in three cats on the heart sensitized by chronic denervation and in two cats on the heart sensitized by cocaine (2 mg./kg.). Both methods of sensitization had the usual effect of equalizing the responses to adrenaline and noradrenaline. Mepyramine caused no further increases in the responses. On the contrary, the addition of more than 2.5 mg./kg. depressed the responses.

The effect of mepyramine upon the responses to cardio-accelerator nerve stimulation was tested in three experiments. In each case mepyramine (5 mg./kg.) increased the responses to supramaximal stimulation; cardio-acceleration was increased from 37 to 48 beats/min. to 56 to 72 beats/min.

With the doses of amine used in this investigation the cardio-acceleration was usually of little more than 90 sec. duration. After administration of the antihistamines, the cardio-acceleration generally lasted not more than 90 to 120 sec. Thus these potentiating drugs did not markedly prolong the chronotropic action of the amines. Subsequent addition of cocaine (2 mg./kg.) extended the duration of cardio-acceleration to 4 to 5 min.

Kuriaki and Uchida (1955) found that diphenhydramine, perfused in a concentration of not less than 1×10^{-5}, caused a slowing of the heart rate in the isolated toad heart. Bradycardia was a constant feature in the action of all five antihistamines in the doses used in the present investigation. Antazoline, diphenhydramine and chlorcyclizine caused a slowing of 10 to 20 beats/min., while mepyramine and promethazine were more active, reducing the rate by 15 to 45 beats/min. The lower basal rate could not be regarded as an explanation for the increased activity of the sympathomimetic amines, for the enhancement was frequently much greater than the degree of slowing. For example, in one experiment diphenhydramine (10 mg./kg.) decreased the basal heart rate from 166 to 146 beats/min. while the cardio-acceleration due to 4 μg. of noradrenaline was increased from 12 to 58 beats/min. Moreover, mepyramine slowed the heart rate by 10 to 40 beats/min. in hearts sensitized by chronic denervation or by cocaine, while it caused no increase in the cardio-accelerator action of the amines.

The Effect of Antihistamines on the Action of Sympathomimetic Amines on the Nictitating Membrane.—In this investigation use was made of information derived from earlier studies of sensitization of the nictitating membrane (Lockett, 1950; Innes and Kosterlitz, 1952, 1954b). Two types of sensitization with different characteristics are known, one typified by the effect of chronic decentralization or of ephedrine, the other by the effect of chronic denervation or of cocaine. In the unsensitized, acutely decentralized or denervated nictitating membrane, adrenaline causes a greater contraction than does noradrenaline. Ephedrine or chronic decentralization brings about an unspecific increase in sensitivity to a wide range of sympathomimetic amines and does not affect the relationship between the sizes of the contractions due to adrenaline and noradrenaline. Cocaine or chronic denervation causes no further increase in adrenaline action but potentiates the action of many other amines bearing a phenolic hydroxyl group in the *meta* position. The action of noradrenaline is thereby increased to equal that of adrenaline. The effect of cocaine or chronic denervation on the action of amines lacking the hydroxyl group in the *meta* position is one of depression. By use of the three amines, adrenaline, noradrenaline and tyramine, the pattern of potentiation can be observed and an attempt made to classify the sensitizing effects of antihistamines into one or other of the types described above.

The effects of mepyramine and diphenhydramine were examined on the nictitating membrane, since these antihistamines gave the most pronounced results on the heart. It was especially important to study the effect of diphenhydramine in view of the likeness of its action to that of cocaine on the heart. The results are summarized in Table II.

TABLE II

POTENTIATION OF THE CONTRACTIONS OF THE NICTITATING MEMBRANE BY ANTIHISTAMINES

Comparison with effects of cocaine, ephedrine, chronic decentralization and chronic denervation. The symbol + refers to an increase in amplitude of contraction and the sign − indicates a decrease in amplitude of contraction. An asterisk refers to the results of Innes and Kosterlitz (1952, 1954b).

Antihistamine	Dose (mg./kg.)	No. of Experiments	Effect on Contractions of Nictitating Membrane Caused by		
			Adrenaline	Noradrenaline	Tyramine
Mepyramine ..	2·5–5	5	+	+	+
Diphenhydramine	5–10	4	+	++	0
Chronic decentralization		*	+	+	+
Ephedrine	0·03–0·1	*	+	+	+
Chronic denervation		*	+	+++	−
Cocaine	2	*	+	+++	−

The potentiation caused by mepyramine followed exactly the pattern of potentiation found in chronic decentralization ; the relationship between the sizes of the contractions was unaltered. This similarity to the effect of chronic decentralization was tested in two experiments with membranes sensitized by chronic decentralization. Mepyramine, in doses which ensured potentiation on the acutely decentralized membrane, caused no alteration of the responses of the chronically decentralized membrane.

Although diphenhydramine potentiated noradrenaline more than adrenaline contractions, a full cocaine-like effect was not attained. Adrenaline and noradrenaline responses were never equalized, while tyramine responses were little altered. Subsequent administration of cocaine showed that the full effect of equalizing adrenaline and noradrenaline action and depressing tyramine action could be produced in these cats. In two experiments with chronically decentralized membranes, diphenhydramine somewhat increased the sensitivity to noradrenaline, but without equalizing the response to adrenaline and noradrenaline. Tyramine responses were slightly reduced, but not to the same extent as after injection of cocaine. Diphenhydramine had no effect on the responses of chronically denervated membranes.

Discussion

In the sensitization of the heart which is exhibited by all five antihistamines examined, the antihistamine potency of the drugs appears to bear no relation to their potentiating activity. Mepyramine, promethazine and chlorcyclizine have been demonstrated by Marshall (1955) to exert greater antihistamine activity than diphenhydramine, while the present observations show diphenhydramine to be the most active in enhancing noradrenaline action.

Inhibition of amine oxidase by diphenhydramine has been demonstrated by Kuriaki and Uchida, who ascribe its sensitizing effect to this property. Amine-oxidase inhibition, however, is unlikely to play a role in the cocaine-like potentiation of sympathomimetic amine action, since Foster, Ing and Varagić (1955) found that no such potentiation on the nictitating membrane and blood pressure was caused by α-cocaine, a structural isomer of cocaine capable of inhibiting amine oxidase as effectively as cocaine.

It does not appear to be possible to link the ability to sensitize the heart with local anaesthetic activity, a property common to antihistamines and many other substances which potentiate sympathomimetic amine action. Diphenhydramine is a more effective local anaesthetic agent than mepyramine (Reuse, 1948), yet mepyramine potentiates adrenaline cardio-acceleration while diphenhydramine does not.

The possibility that two separate, and as yet unknown, mechanisms of sensitization are involved is suggested by the fact that diphenhydramine, like cocaine and chronic denervation, sensitizes the heart to noradrenaline but not to adrenaline action, while the other antihistamines sensitize the heart to the action of both amines.

The observations of the effect of diphenhydramine on the nictitating membrane agree with the work of Lecomte and Fischer (1952) showing that amines possessing a phenolic hydroxyl group in the *meta* position are potentiated by antihistamines. With mepyramine, however, the absence of the hydroxyl group in this position is not critical, as the action of tyramine was enhanced by this drug in each of five experiments in the present series. Thus mepyramine and diphenhydramine sensitization fall into different categories. Mepyramine causes an unspecific sensitization similar to the increased sensitivity found after ephedrine or chronic decentralization ; potentiation by diphenhydramine, on the other hand, more closely follows the sensitization caused by cocaine or chronic denervation, as it does in the heart.

In view of the existence of two distinct patterns of sensitization of the nictitating membrane it seems impossible to account for the phenomena of sensitization plausibly in terms of a single mechanism.

It appears therefore that two sensitizing mechanisms must be sought, one of which potentiates the actions of all sympathomimetic amines, 5-hydroxytryptamine (Innes and Kosterlitz, 1954b) and acetylcholine (Cannon and Rosenblueth, 1936), while the second further potentiates the actions of some sympathomimetic amines but suppresses the actions of others.

I am indebted for generous gifts of Antistin to Ciba Laboratories Ltd., of Benadryl to Parke, Davis and Co. Ltd., of chlorcyclizine to Burroughs Wellcome and Co., and of Phenergan to May and Baker Ltd.

References

Cannon, W. B., and Rosenblueth, A. (1936). *Amer. J. Physiol.*, **116**, 408.

Foster, R., Ing, H. R., and Varagić, V. (1955). *Brit. J. Pharmacol.*, **10**, 436.

Innes, I. R., and Kosterlitz, H. W. (1950). *J. Physiol.*, **111**, 18*P*.

—— —— (1951). *Brit. J. Pharmacol.*, **6**, 651.

Innes, I. R., and Kosterlitz, H. W. (1952). *J. Physiol.*, **118**, 28*P*.
—— —— (1954a). Ibid., **124**, 17.
—— —— (1954b). Ibid., **124**, 25.
Kosterlitz, H. W., Krayer, O., and Matallana, A. (1955). *J. Pharmacol.*, **113**, 460.
Kuriaki, K., and Uchida, T. (1955). Ibid., **113**, 228.
Lecomte, J., and Fischer, P. (1952). *Arch. int. Pharmacodyn.*, **91**, 79.
Lockett, M. F. (1950). *Brit. J. Pharmacol.*, **5**, 485.
Marshall, P. B. (1955). Ibid., **10**, 270.
Reuse, J. J. (1948). Ibid., **3**, 174.
Sherrod, T. R., Loew, E. R., and Schloemer, H. F. (1947). *J. Pharmacol.*, **89**, 247.
Walter, W. G., and Ritchie, A. E. (1945). *Electron. Eng.*, **17**, 585.
Yonkman, F. F., Chess, D., Mathieson, D., and Hansen, N. (1946). *J. Pharmacol.*, **87**, 256.