Brit. J. Pharmacol. (1955), **10**, 186.

MODIFICATION BY DRUGS OF THE RESPONSE OF THE RAT'S UTERUS TO ADRENALINE

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(RECEIVED DECEMBER 20, 1954)

When adrenaline is assayed by its inhibitory action on the contractions of the rat's uterus elicited by carbachol, the question of the specificity of the inhibition often arises. This happens particularly when impure extracts of biological material are examined. Thus it would be useful to have a specific antagonist of this reaction which could be employed in a way analogous to the use of mepyramine in assays of histamine. A second problem arises in the assay of very small quantities of adrenaline, namely the unpredictability of the sensitivity of the uterine tissue, so that some specimens have to be discarded owing to their high threshold. In the following paper a number of drugs were tested for their suitability either as antagonists or as sensitizers for the inhibitory action of adrenaline on uterine contraction.

In the search for suitable drugs the fact was borne in mind that drug antagonists may, in very small doses, act as sensitizers to the agonist. Thus Gaddum and Kwiatkowski (1938) confirmed and amplified the evidence that this is true of ephedrine. This observation was extended by Jang (1941) to adrenaline antagonists which were chemically less nearly related to adrenaline than is ephedrine. Jang examined the influence of ergotoxine, yohimbine and piperidyl-benzodioxane on several motor effects of adrenaline and found that one of them, the constriction of the vessels in the rabbit's ear, was enhanced by small doses of either of the three drugs. Inhibitory actions of adrenaline were not investigated.

METHODS

Uterine horns of non-pregnant rats (body weight 120–250 g.) were suspended in a 2 ml. bath containing an aerated salt solution of low calcium content and were stimulated by carbachol at regular intervals (for details see Gaddum, Peart, and Vogt, 1949; Gaddum and Lembeck, 1949). In some experiments both horns were suspended simultaneously in two baths immersed in the same thermostat and the carbachol contractions were recorded on the same drum. The drug under investigation was added to one bath, and any effects on the sensitivity to adrenaline compared with the responses of the untreated horn. Spontaneous changes in sensitivity to adrenaline could thus be distinguished from those produced by the drug.

Most drugs were dissolved in 0.9% NaCl. Exceptions were the members of the halo-alkylamine group (Table II, Nos. 19–21), of which 0.1% stock solutions in propylene glycol were prepared and diluted with 0.9% NaCl immediately before use; "Dibozane" (Table I, No. 3), jervine, pseudojervine, and veratramine, which were dissolved in 3% acetic acid to make 1% or 0.1% solutions; these also were diluted with saline immediately before use; veratrosine, which was dissolved in 0.9% NaCl to which one drop of 0.1N-HCl was added for each 5 ml. of solution; and β-tetrahydronaphthylamine carbonate, 5-benzyloxygramine and "Medmain" (Table II, No. 26), which were dissolved in HCl before dilution with 0.9% NaCl; they were neutralized with $NaHCO_3$ immediately before use.

Two ways were used of exposing the uterus to the action of the drugs under investigation. One was to add the drug to the reservoir of salt solution which did not contain the carbachol; in this way exposure to the drug was continuous except for the 50 sec. in each two-minute cycle in which the muscle was contracting under the influence of carbachol. The other was to add the drug to the bath itself while the flow of salt solutions from the automatic machine was interrupted and to leave it in for (usually) 10 min. The bath was then washed and the normal cycle of carbachol-containing and carbachol-free salt solutions resumed. This short exposure is particularly suited for compounds the action of which is only slowly reversible.

RESULTS

Table I shows that, of the 27 drugs examined, 18 had no effect on the inhibition of uterine contractions by adrenaline. The table gives only the maximal concentrations in the bath, or in the reservoir feeding the bath, tolerated by the uterus; further increase in the dose produced irregularities or lack of response to the carbachol stimulus. Thus chlorcyclizine and chlorpromazine could only be employed in relatively low concentrations, as they have a fairly strong anti-acetylcholine action.

TABLE I

DRUGS WHICH DID NOT MODIFY THE INHIBITORY ACTION OF ADRENALINE ON THE RAT'S UTERUS

No.	Compound	Maximal Concentration (mg./l.) Applied to Tissue	
		For 10 min.	Permanently
1	Diethylamino-methyl-benzodioxane HCl (883 F)	50	1·0
2	Piperidyl-methyl-benzodioxane HCl (933 F)	5	
3	1, 4 bis (1, 4-benzodioxane-2-methyl)-piperazine ("Dibozane")		10
4	Thymoxyethyldiethylamine HCl (929 F)	0·5	
5	Phenindamine hydrogen tartrate ("Thephorin")	0·5	
6	Chlorprophenpyridamine maleate ("Chlortrimeton")	50	1·0
7	Chlorcyclizine HCl ("Histantin")	0·005	
8	Chlorpromazine HCl ("Largactil")	0·25	1·0
9	Yohimbine HCl	50 (5 min.)	
10	Tolazoline HCl ("Priscol")	0·5	2·5
11	Phentolamine ("Rogitine")	25	0·25
12	Jervine (Veratrum alkaloids)	5	
13	Pseudojervine (Veratrum alkaloids)	0·25	
14	Veratrosine		5
15	Ephedrine HCl	5	
16	Iproniazid ("Marsilid")	0·5	10
17	Eserine sulphate		2
18	NaF	100	1,000 (30 min.*)

* After that time responses to carbachol irregular.

TABLE II

DRUGS WHICH MODIFIED THE INHIBITORY ACTION OF ADRENALINE ON THE RAT'S UTERUS

No.	Compound	Effective Concentrations, Applied to Tissue for 10 min. (mg./l.)	Threshold to	
			Adrenaline	Isoprenaline
19	Dibenamine HCl	0·05–0·5	Lowered	Lowered
20	*N*-(9 fluorenyl)-*N*-ethyl-β-chlorethylamine HCl (SKF 501)	0·05	,,	,,
21	*N*-benzyl-*N*-(β-phenoxyisopropyl)-β-chlorethylamine HCl (SKF 688A, "Dibenzyline")	0·037–0·25	,,	,,
22	Dihydroergotamine methanesulphonate (DHE)	0·5–5·0	Raised	Unchanged
23	Lysergic acid diethylamide (LSD)	0·01–20·0	,,	,,
24	β-Tetrahydronaphthylamine carbonate (β-Tetra)	0·1–2·0	Lowered	Lowered
25	5-Benzyloxygramine	0·012–0·37	,,	Unchanged or lowered
26	2-Methyl-3-ethyl-5-dimethylamino-indole("Medmain")	5·0*	Raised	Raised
27	Veratramine	0·005–0·01	Temporarily raised	Unchanged

* Added for 70 sec. in a 2 min. cycle.

Many experiments were done with concentrations lower than those listed, and no effect of the drugs was observed.

Table II contains those drugs which produced some modification of the inhibitory effect of adrenaline on contractions due to carbachol. The group of the halo-alkylamines (Nos. 19–21) sensitized to the action of adrenaline. Application of low concentrations of any of these drugs (e.g., 0.037 mg. dibenzyline/l.) for 10 min. was usually effective; prolonged exposure to the same concentration caused interference with the response to carbachol; if this concentration was reduced to one-half, it was tolerated for a longer period, but produced a lesser degree of sensitization. On theoretical grounds one might have expected an antagonism to the action of adrenaline by larger doses of the same drugs. When, however, concentrations from 0.25 mg./l. onwards were applied, even very briefly, the uterine responses to carbachol were suppressed so that their inhibition by adrenaline could not be investigated.

These halo-alkylamines have proved very useful in solving the practical problem of rendering uteri of low sensitivity to adrenaline suitable for the assay of fractions of a nanogram (Holzbauer and Vogt, 1954). There were only a few occasions when sensitization of the uterus was not obtained by one of these drugs. Of the three compounds tested, dibenzyline appeared to be the most effective, though not enough experiments were carried out with the other two compounds to establish the significance of the difference. Fig. 1 shows a tracing of a 20-fold increase in sensitivity to adrenaline by the addition, to a 2 ml. bath, of 0.05 followed by 0.075 μg. dibenzyline, each dose left in contact with the tissue for 10 min. The tracing also shows that the sensitization affects isoprenaline and adrenaline equally. Experiments on uteri with varying initial sensitivities suggested that insensitive uteri showed a greater lowering of the threshold than sensitive ones, and that responses to less than 0.05 ng. adrenaline (per 2 ml. bath) were unobtainable; it is of interest that this maximal sensitivity which may be obtained artificially is the same as the highest sensitivity occurring naturally in some specimens.

A second group of drugs found to modify the inhibitory action of adrenaline consists of three substances related to the ergot alkaloids. Two of these, dihydro-ergotamine (DHE) and lysergic acid diethylamide (LSD), reduced the inhibitory effect of adrenaline, LSD being more specific than DHE, in that it diminished the sensitivity to adrenaline to between 1/5 and 1/100 of its previous value without interfering with the carbachol contractions; DHE began to inhibit the response to carbachol when the sensitivity to adrenaline was reduced to about one-half. The action of both

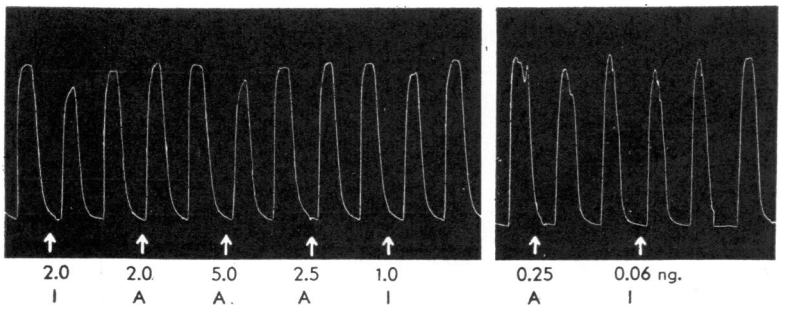

FIG. 1.—Rat uterus. Contractions elicited every 2 min. by carbachol. At the arrows, different doses of adrenaline (A) or isoprenaline (I) added to the 2 ml. bath 45 sec. before the next dose of carbachol is due. Left: sensitivity before dibenzyline. Right: sensitivity after 0.05 μg. dibenzyline added to the bath for 10 min., followed 40 min. later by 0.075 μg. dibenzyline for 10 min. Tracing starts another 24 min. later. Threshold to adrenaline and to isoprenaline lowered approx. 20 times. Doses in nanograms.

substances was specific in the sense that the response to isoprenaline was less affected than that to adrenaline. Fig. 2 is an example of a reduction by LSD of the sensitivity to adrenaline to 1/8 of its original value without any change in the response to isoprenaline. The degree of discrimination between adrenaline and isoprenaline varied from one uterus to the other; when the discrimination is good, such a uterus is a useful tool in helping to decide whether an inhibition is caused by adrenaline or by isoprenaline, a question which has arisen in the assay of adrenal medullary extracts (Lockett, 1954). The doses required to abolish the responses to adrenaline varied between 0.01 and 20 mg./l., depending on the amount of adrenaline to be antagonized. Neither LSD nor DHE sensitized to the action of adrenaline when applied in lower concentrations (0.005–0.05 mg. DHE/l.; 0.005 mg. LSD/l.).

The third substance from this group, β-tetrahydronaphthylamine ("β-Tetra," No. 24), did not raise but lowered the threshold to adrenaline. The sensitization obtained in a uterus responding initially to 0.25 ng. was about fourfold, and was thus no less than that produced by the haloalkylamines. When the concentration was increased above 2 mg./l., the threshold to adrenaline rose again without ever exceeding the original level. Increase in the concentration of β-Tetra above 40 mg./l. interfered with the effects of carbachol, so that the failure to demonstrate any antagonism to adrenaline may have been due to the limitation in the concentration imposed by the impairment of the response to carbachol. β-Tetra affected adrenaline and isoprenaline to the same extent.

The next two substances tested, 5-benzyloxygramine and medmain, are 5-hydroxytryptamine (HT) analogues, both endowed with an antagonistic action to HT on the rat's uterus (Gaddum, Hameed, Hathway, and Stephens, 1955; Shaw and Woolley, 1954). Benzyloxygramine (No. 25) lowered and medmain (No. 26) raised the threshold to adrenaline. Very high concentrations were required to obtain any effect with medmain, and the effects were rapidly reversible. This substance has the peculiarity of being rapidly oxidized in an aerated salt solution (Woolley, personal communication). It was found that the only effective way of using it was to add it to the organ bath as soon as the carbachol was being washed out and to leave it in contact with the tissue for 70 sec., when the next dose of carbachol was due. When this was done repeatedly, the organ was exposed to the drug for 70 sec. in every 2 min. cycle. When tested in this way in the presence of medmain (5 mg./l.), threshold doses of adrenaline and of isoprenaline were antagonized, whereas the response to larger doses was little changed. When more medmain was used, antagonism to the response to carbachol ensued.

In contrast to the antagonistic effect of medmain, 5-benzyloxygramine sensitized to adrenaline, whilst the response to isoprenaline was not consistently

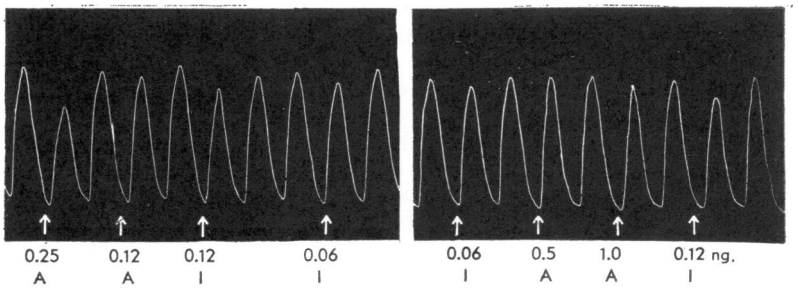

FIG. 2.—Rat uterus. Contractions elicited every 2 min. by carbachol. At the arrows, different doses of adrenaline (A) or isoprenaline (I) added to the 2 ml. bath 45 sec. before the next dose of carbachol is due. Left: sensitivity before LSD. Right: sensitivity after 2.5 μg. LSD added to the bath for 10 min., followed 15 min. later by 5 μg. LSD for 10 min. Tracing starts another 12 min. later. Threshold to adrenaline raised by a factor of 8; threshold to isoprenaline unchanged. Doses in nanograms.

enhanced. It was the only substance encountered which sometimes sensitized to adrenaline without sensitizing to isoprenaline. The effective concentrations varied greatly in different preparations, and so did the maximal concentrations which left the responses to carbachol unimpaired. Inhibition, by higher concentrations, of the response to adrenaline was not observed, possibly because of the limit imposed by the interference of the drug with the stimulation by carbachol. The smallest effective dose of adrenaline after 5-benzyloxygramine was 0.12 ng./2 ml. bath, and thus not much different from that achieved with the halo-alkylamines. For practical purposes the great variability of the antagonism of 5-benzyloxygramine to carbachol would make it a difficult drug to employ in assays.

The last substance found to affect the inhibitory action of adrenaline on the rat's uterus was veratramine. In uteri which had a low threshold to adrenaline, small doses of the drug (0.005–0.01 mg./l.) acted as antagonists and raised the threshold between 2- and 13-fold. The threshold to isoprenaline remained unaltered. The antagonistic effect was, however, evanescent, and disappeared either spontaneously or when larger doses of veratramine were added (0.05–0.5 mg./l.). It was not observed when the initial dose of veratramine was high; in fact, the larger doses were occasionally seen to sensitize the uterus to the action of adrenaline. However, when the movements of both horns of one uterus were recorded simultaneously and the drug was added to one horn only, so that spontaneous changes of threshold could be taken into account, the sensitization was not obtained consistently.

The drug precipitates slowly when its acid solution is diluted with physiological salt solutions. Therefore, each time a dose was added to the organ bath, it was freshly prepared from the acidified stock solution. It could be seen gradually to precipitate in the bath, and prolonged exposure of the tissue to the drug by adding it to the reservoir containing the salt solution was obviously not possible. It may be that the complex and erratic effects of veratramine on the uterine responses to adrenaline are due to the physical instability of its neutral solutions.

Discussion

A surprising result of these experiments is the fact that, in spite of the existence of so many adrenaline antagonists, the inhibition exerted by adrenaline on the carbachol contractions of the rat's uterus proved very difficult to abolish. This is only partly due to the fact that inhibitory responses to adrenaline are less easily abolished than motor responses, and mainly to the relative lack of specificity of most adrenaline antagonists, which is shown in this preparation by an interference with the response to carbachol before or simultaneously with an interference with the response to adrenaline.

Of the 27 substances tested, nine were found to have an effect on the inhibition of uterine contractions exerted by adrenaline. It is striking that all nine substances were "specific" antagonists of HT on the rat's uterus in the sense that they inhibited HT in concentrations which had no effect against choline esters. This was known for the halo-alkylamines, for DHE and LSD, and for the two HT analogues Nos. 25 and 26 (Gaddum and Hameed, 1954; Gaddum *et al.*, 1955; Fingl and Gaddum, 1953; Gaddum, 1953; Shaw and Woolley, 1954). We found it to be also true of β-Tetra, of which 10 mg./l., and of veratramine, of which doses ranging from 0.01 mg./l. to 0.5 mg./l., raised the threshold to HT, whilst the response to carbachol was hardly changed.

None of the substances which failed to modify the action of adrenaline on the uterus (Table I) is known to antagonize HT specifically. Gaddum and Hameed (1954) failed to demonstrate that either yohimbine or 933 F were specific antagonists of HT, but data for the remaining substances of Table I are unfortunately lacking.

The three halo-alkylamines tested were very similar in their action: a sensitization was obtained with sufficient regularity to be of practical use, provided the doses were kept small so as to avoid interference with the response to carbachol. The limit thus imposed on the dosage made it impossible to decide whether larger doses might have antagonized the action of adrenaline. The position was similar for the other two drugs found to sensitize towards adrenaline, β-Tetra and 5-benzyloxygramine; the only indication that large doses might have an effect opposite to that of small doses was obtained with β-Tetra, large doses of which abolished the sensitization produced by small doses. The final sensitivity achieved was of the same order for all drugs and never exceeded the highest naturally occurring sensitivity to adrenaline.

The three compounds related to ergot alkaloids were much less uniform in their action than the halo-alkylamines. Two of them (DHE and LSD) were adrenaline antagonists, whereas the third, β-Tetra, sensitized to adrenaline. The antagonism was more clearly seen in LSD than in DHE, probably because LSD, in contrast to DHE, reduced

the response to adrenaline long before affecting the contractions due to carbachol. The effect of both substances was specific in the sense that adrenaline was inhibited at a dosage which left isoprenaline unaffected. Both substances, nevertheless, antagonized HT more readily than adrenaline.

Of the two HT-analogues, 5-benzyloxygramine increased and medmain decreased the sensitivity to adrenaline. The antagonistic effect of medmain was only obtained with large doses and may possibly be of a completely different nature from that of DHE and LSD. An indication that a different phenomenon might be involved lies in the fact that the response to adrenaline and isoprenaline was affected equally by medmain, whereas adrenaline only was inhibited by DHE and LSD.

The action of veratramine, ill-defined as it is, is of some interest in view of the specific antagonism of that substance towards the tachycardia produced by adrenaline (Krayer, 1949).

Amongst the inactive substances, there were such recognized adrenaline antagonists as the benzodioxanes, including the very potent new compound "Dibozane" (Leitch, Liebig, and Haley, 1954), two antihistamines known to be powerful anti-adrenalines (929 F and phenindamine; Bovet and Bovet-Nitti, 1948), and the imidazolines tolazoline and phentolamine. The lack of sensitization obtained by the anti-amine-oxidases ephedrine and iproniazid, the latter being very powerful *in vitro* (Zeller, Barsky, Fouts, Kirchheimer, and Van Orden, 1952) and *in vivo* (Schayer, 1953), shows that the intensity of the response of the isolated uterus to adrenaline is not affected by the activity of amine oxidase in the tissue. Eserine was investigated because Agar (1940) had shown that the inhibitory effect of adrenaline on the guinea-pig's uterus is reversed by pretreating the tissue with eserine. Sodium fluoride was examined on account of the theory put forward by Mohme-Lundholm (1953) that adrenaline acts on the tissues by producing lactic acid, and that NaF, by prohibiting glycolysis, prevents the action of adrenaline. No confirmation of this view was obtained.

A very obvious conclusion from this investigation is the complete unpredictability of the effects which even chemically related drugs exert on the inhibitory effect of adrenaline on uterine muscle.

Summary

1. Twenty-seven substances were tested for their power to modify the inhibition by adrenaline of the carbachol contractions of the rat's uterus.

2. The halo-alkylamines, and more particularly dibenzyline, sensitize the rat's uterus to adrenaline and isoprenaline and are useful in assays of very small quantities of these compounds.

3. Lysergic acid diethylamide and dihydroergotamine antagonize the inhibitory action of adrenaline on the rat's uterus. Isoprenaline is less affected than adrenaline, so that, in a favourable preparation, this test will distinguish adrenaline from isoprenaline.

4. β-tetrahydronaphthylamine and 5-benzyloxygramine increased, and medmain decreased, the sensitivity of the rat's uterus to adrenaline; the effects of medmain were too small to be of much practical use. 5-Benzyloxygramine was the only substance encountered which sensitized more consistently to adrenaline than to isoprenaline.

One of us (M. V.) is indebted to the Medical Research Council for a grant towards the expenses of this work. Part of the work was done during the tenure by one of us (M. H.) of a British Council Bursary. Our thanks are due to the following for samples of drugs: to Messrs. Rhône-Poulenc, Paris, for compounds 833 F and 929 F (2214 RP) which we obtained through the courtesy of Dr. R. Wien of Messrs. May and Baker; to Messrs. Smith, Kline and French, Philadelphia, for the three halo-alkylamines; to the Schering Corporation, Bloomfield, New Jersey, for chlorprophenpyridamine maleate; to Glaxo Laboratories, Ltd., for 5-benzyloxygramine; to Dr. O. Krayer, Harvard Medical School, for samples of the veratrum alkaloids; to Dr. Th. J. Haley, Los Angeles, for Dibozane; and to Dr. D. W. Woolley, New York, for medmain.

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