Biochemical Pharmacology, 1964, Vol. 13, pp. 685–690. Pergamon Press Ltd., Printed in Great Britain.

THE BIPHASIC EFFECT OF PHENOTHIAZINES AND RESERPINE ON THE RELEASE OF EPINEPHRINE FROM ADRENOMEDULLARY GRANULES AND ITS DEPENDENCE ON pH*

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(*Received* 5 *December* 1963; *accepted* 2 *January* 1964)

Abstract—The effects of trifluoperazine, triflupromazine, chlorpromazine, and reserpine on the release of epinephrine from adrenomedullary granules *in vitro* were investigated at various pH values ranging from 5·6 to 8·4. In acid medium the drugs, at concentrations of 10^{-5} to 10^{-4} M, delayed the rate of spontaneous release, whereas in alkaline medium the same concentrations intensified release. The phenothiazine drugs did not inhibit release in alkaline medium at any concentration, whereas 10^{-6} M reserpine, at pH 8·0, did.

At a pH of 6 or below and of 8 or above, the effects were similar whether granules were suspended in a sucrose or an electrolyte medium. However, at pH 7·2, phenothiazines enhanced release in an electrolyte medium and delayed it in a sucrose medium.

A possible mechanism of the effects is proposed.

WHEN the effect of a series of drugs on the release of epinephrine from adrenomedullary granules was tested *in vitro* in our laboratory, two groups stood out as particularly active: reserpine and the phenothiazines, especially those with tranquilizing activity.[1] Although these drugs greatly accelerated the rate of release at concentrations of about 10^{-4} M, they had the opposite effect at concentrations of the order of 10^{-6} to 10^{-5} M. A biphasic effect of this kind was first described by von Euler and Lishajko[2] for reserpine.

Further study has now shown that the two phases of action, inhibitory and stimulatory, can largely be separated by variation of the pH. In a slightly alkaline medium (pH about 8) the rate of release is accelerated, whereas at a pH of about 6 inhibition of release predominates.

EXPERIMENTAL

The preparation of homogenates of rabbit adrenal glands (600 *g* supernatant fraction), the incubation and the final high-speed centrifugation were as previously described,[1] unless stated otherwise.

In experiments with reserpine, analysis was carried out as previously described.[1] Since the phenothiazines used did not interfere with the estimation of epinephrine, passage of the solutions through columns of alumina and cation-exchange resin was omitted. In these cases the high-speed residues were resuspended in water; 2 ml

* The principal results of this study were presented at the Second International Pharmacological Meeting in Prague, 20–23 August 1963.

of 10% metaphosphoric acid was added to both residue and supernatant fractions, and water was added to a total volume of 10 ml. The samples were centrifuged and the clear solutions adjusted to pH 6·0. Aliquots of 2 and 1 ml of the residue and supernatant fractions, respectively, were used for the estimation of epinephrine by the ethylenediamine method.[3] Since we had previously found,[1] in agreement with other investigators, that rabbit adrenals contain little norepinephrine, the fluorescence intensity at 520 mμ (excitation: 420 mμ) was taken as a measure of epinephrine concentration.

Suspension media. 'Sucrose medium' consisted of 0·3 M sucrose (95 vol) and isotonic buffer (5 vol). 'KCl medium' contained 0·164 M KCl and 0·002 M $MgCl_2$ (90 vol) and isotonic buffer (10 vol).

The following approximately isotonic buffers were used for the range of pH 6·0 to 8·0: 0·17 M NaH_2PO_4 and 0·123 M Na_2HPO_4, mixed to give the required pH.

For pH > 8·0: 0·2 M Tris + 0·16 N HCl, mixed to give the required pH.

For pH > 6·0: 0·15 M sodium acetate and 0·3 M acetic acid, mixed to give the required pH.

RESULTS

Spontaneous release

Each experiment included drug-free controls, in duplicate or triplicate, for the estimation of the initial and final concentrations of epinephrine. The rate of release was calculated from the concentration changes of epinephrine in the particulate fraction, although the soluble fraction was also analyzed. As a rule, decreases in the particulate fraction were satisfactorily accounted for by increases in the soluble fraction.

The rate of release was expressed in terms of the half-life of particle-bound epinephrine. This procedure rests on the assumption that the release of epinephrine is a first-order reaction. Figure 1 shows that the process is in reality more complicated,

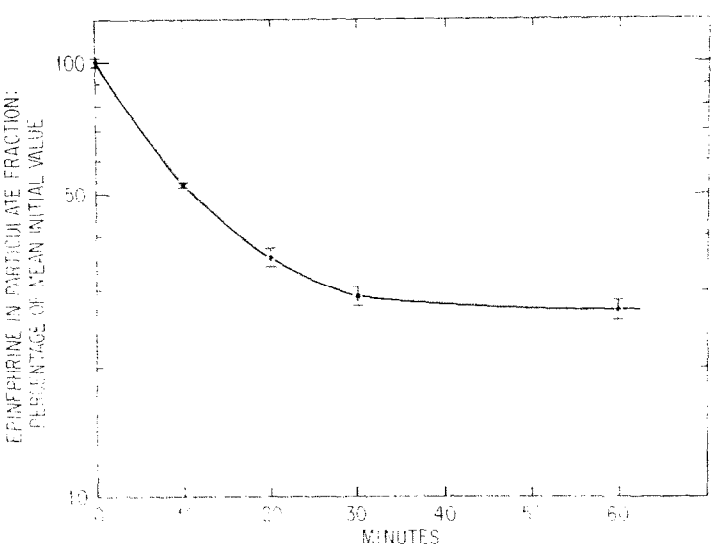

FIG. 1. Spontaneous release of particle-bound epinephrine. KCl–phosphate medium, pH 7·0, 37°. Means of triplicate experiments, ± SEM.

such as might be expected from a heterogeneous population of granules. Nevertheless, the course of release for the first 70% of bound epinephrine approximates first-order kinetics; only in some instances did depletion exceed 70%.

A measure of the experimental error of spontaneous release estimates was obtained by calculating the percentage standard deviation from the mean of 15 duplicate, 6 triplicate and 1 quadruplicate estimation of the half-life of particle-bound epinephrine in the absence of drugs.[4] It amounted to 14·10% (30 degrees of freedom). Deviations from the control of more than 28% may therefore be regarded as significant at the 5% level of probability.

Effect of phenothiazines on the rate of release

Some typical results are shown in Figs. 2–4 and Table 1. Although the examples shown represent single experiments, each experiment was repeated at least twice with essentially similar results. In the diagrams, the half-life of particle-bound epinephrine, expressed as a percentage of the half-life of epinephrine in a drug-free control medium, has been plotted against log drug concentration. The 100% line therefore indicates the rate of spontaneous release; points above 100 indicate inhibition, points below 100 acceleration of release. The shaded band covers the area limited by twice the standard deviation of the control.

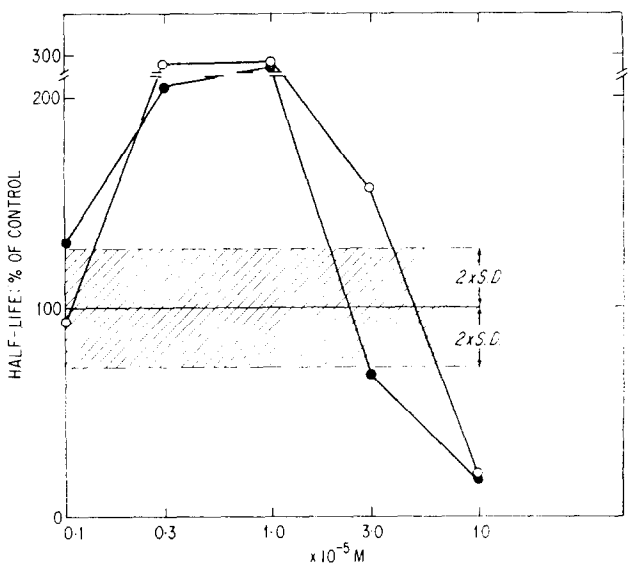

FIG. 2. Effects of trifluoperazine (●—●) and triflupromazine (○—○) on the release of particle-bound epinephrine. KCl–phosphate medium, pH 6·7, 30°, 30 min.

An example of the biphasic effect of two phenothiazines, trifluoperazine, and triflupromazine, is shown in Fig. 2. The experiment was carried out in KCl medium at pH 6·7. At concentrations of 0·3 to 1 × 10^{-5} M the two drugs inhibited release; they accelerated it when the concentration was increased to 10^{-4} M.

When the pH of the incubating medium was lowered to 5·6, the inhibitory effect of trifluoperazine was still in evidence at a concentration of 3×10^{-5} M (Fig. 3), that of triflupromazine at a concentration of 10^{-4} M (Table 1). Even when the concentration of trifluoperazine was increased to 10^{-3} M, the releasing effect was less marked than that observed at pH 6·7 at a concentration of 10^{-4} M.

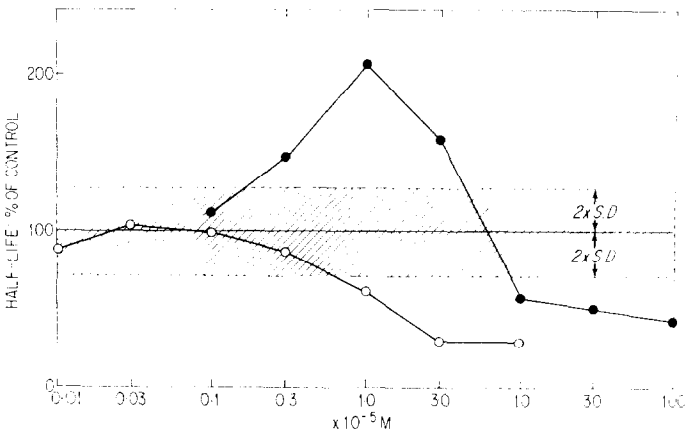

FIG. 3. Effect of trifluoperazine on the release of particle-bound epinephrine (37°, 30 min). In sucrose–acetate buffer, pH 5·6 (●–●). In sucrose–Tris buffer, pH 8·0 (○—○).

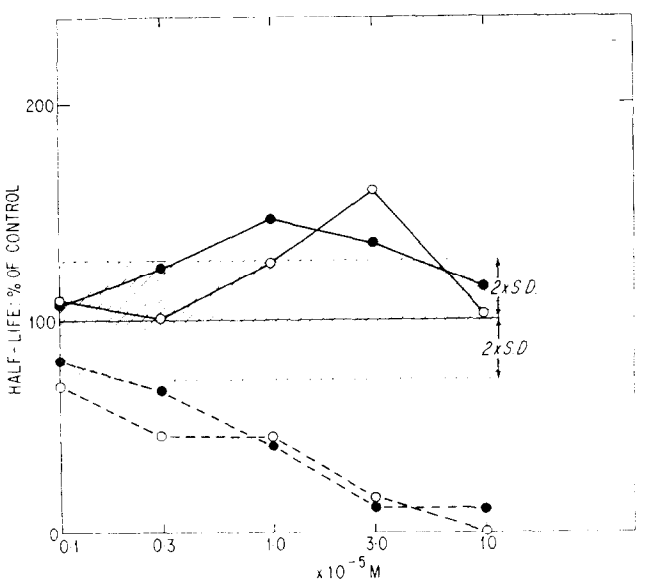

FIG. 4. Effects of trifluoperazine (●—●) and triflupromazine (○—○) on the release of particle-bound epinephrine (37°, 30 min). Solid lines: sucrose medium, pH 7·2. Broken lines: KCl medium, pH 7·2.

On the other hand, raising the pH to 8·0 or 8·4 completely abolishes the inhibitory effect of the two drugs over an extended range of concentrations. Instead, at concentrations $> 10^{-5}$ M, the rate of release is increased. Concentrations of $0{\cdot}3 \times 10^{-5}$ M and below are without effect.

Similar observations were made with chlorpromazine (Table 1).

TABLE 1. THE EFFECT OF DRUGS ON THE RELEASE OF EPINEPHRINE FROM ADRENOMEDULLARY GRANULES

Incubation for 30 min at 37° (sucrose medium) or 30° (KCl medium)

Drug	M × 10^{-5}	Medium	pH	Half-life (% of control)
Trifluprομazine	0·3	Sucrose	5·6	177·5*
	1·0			266·1*
	3·0			252·2*
	10·0			290·0*
	30·0			64·9*
	0·1	Sucrose	8·4	86·9
	0·3			79·5
	1·0			50·3*
	3·0			21·8*
	10·0			12·8*
Chlorpromazine	0·3	Sucrose	5·6	118·6
	1·0			91·3
	3·0			105·0
	10·0			185·0*
	30·0			51·1*
	0·1	Sucrose	8·4	79·2
	0·3			105·1
	1·0			81·2
	3·0			62·5*
	10·0			45·4*
Reserpine	0·1	KCl	6·0	156·0*
	0·3			143·4*
	1·0			179·1*
	3·0			305·0*
	10·0			305·0*
	0·1	KCl	8·0	265·4*
	0·3			85·8
	1·0			65·5*
	3·0			62·0*
	10·0			35·4*
Trifluoperazine	0·1	KCl	6·0	191·1*
	0·3			300·4*
	1·0			409·3*
	3·0			101·9
	10·0			94·8
Trifluprομazine	0·1	KCl	6·0	152·3*
	0·3			117·9
	1·0			360·2*
	3·0			107·0
	10·0			102·3
Trifluoperazine	0·1	KCl	8·0	90·8
	0·3			80·0
	1·0			50·5*
	3·0			40·7*
	10·0			28·6*

* Values significantly different from control.

Effects of the medium

Differences dependent on the composition of the medium have previously been noted:[1] generally, an electrolyte medium proved more favorable for the demonstration of releasing activity, whereas a sucrose medium enhanced inhibition. It now appears that effects are essentially the same in both types of media, as long as the reaction is sufficiently alkaline or acid, but differences are observed near the neutral point. Thus, at pH 7·2, trifluoperazine and triflupromazine intensify release in an electrolyte medium, whereas they are mainly inhibitory in a sucrose medium (Fig. 4), in agreement with our earlier observations.

The effect of reserpine

The effect of reserpine on the rate of release depends on pH in the same way as that of the phenothiazines: concentrations from 10^{-5} to 10^{-4} M cause inhibition of release in acid solution and acceleration of release in alkaline solution (Table 1). There is one difference, however: inhibition was observed in alkaline medium at a concentration of 10^{-6} M, whereas phenothiazines did not produce inhibition in alkaline medium at any concentration.

DISCUSSION

The data at hand are insufficient for a detailed theoretical interpretation, but they invite speculation as to a possible mechanism. It might be suggested that the cationic species of the drug is responsible for inhibition and the un-ionized base for acceleration of release. One would expect the uncharged base to be more lipid soluble and therefore to penetrate more easily into or through the membrane, thus possibly impairing its structure or its function, whereas the more hydrophilic cation would attach itself to the surface or the pores of the membrane and thereby impede the egress of solutes.

The affinity of the cation to the membrane may be due in part to electrostatic attraction, and one can imagine this process to be inhibited by competition of other charged particles, which would account for the difference between sucrose and electrolyte media.

The mechanism proposed does not explain why reserpine at low concentrations inhibits release even in alkaline medium. Possibly reserpine in the form of the un-ionized base acts on the membrane in such a way that the transport of solutes is inhibited before membrane function breaks down completely. Undoubtedly, reserpine (pK 6·6[5]) is a much weaker base than the phenothiazines used, and even at pH 6 the uncharged form will account for a significant proportion of the drug.

Acknowledgements—We are grateful to Miss Alzena Graves for careful technical assistance.

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