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THE INFLUENCE OF DESMETHYLIMIPRAMINE ON THE CHRONOTROPIC RESPONSE TO ENDOGENOUS AND EXOGENOUS NORADRENALINE IN THE ISOLATED ATRIA

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Desmethylimipramine, a metabolite of imipramine (Herrmann & Pulver, 1960) through which the activity of imipramine is mediated (Sulser, Watts & Brodie, 1962), is currently of considerable interest because it potentiates the actions of noradrenaline on various adrenergic effector organs (Schaeppi, 1960 ; Sigg, Soffer & Gyermek, 1963). The activity of the drug in sensitizing adrenaline receptors to the action of catecholamines has been postulated to result from an inhibition of uptake of the amines by sympathetic nerve terminals (Iversen, 1965 ; Titus, Matussek, Spiegel and Brodie, 1966), thus permitting higher concentrations of the amines to reach and activate the adrenaline receptors (Furchgott, Kirpekar, Reiker & Schwab, 1963). A similar sensitization of receptors and inhibition of the amine uptake has long been recognized in preparations treated with cocaine (MacMillan, 1959 ; Van Zwieten, Widhalm & Hertting, 1965 ; Hardman, Mayer & Clark, 1965 ; Bhagat, Bovell & Robinson, 1967). Responses to tyramine of the heart, the blood pressure and the nictitating membrane are inhibited by cocaine (Fleckenstein & Stöckle, 1955 ; Trendelenburg, 1961), whereas the responses to sympathetic nerve stimulation are potentiated (Moore, 1966). Little is known, however, about the actions of desmethylimipramine on atrial responses to the application of directly and indirectly acting sympathomimetic amines and to cardiac noradrenaline released by sympathetic stimulation in isolated preparations.

The present study concerns (1) modifications by desmethylimipramine of the positive chronotropic response of isolated atria to noradrenaline, tyramine and sympathetic nerve stimulation, and (2) comparison of the actions of desmethylimipramine, cocaine and procaine on the responses to cardiac noradrenaline.

METHODS

Fifty-nine albino rabbits of either sex, 1.8 to 2.2 kg body weight, were used. Under ether anaesthesia both sympathetic nerves, vagi and common carotid arteries were dissected free from

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surrounding tissues along the cervical trachea and oesophagus. The animals were killed by cutting the common carotid arteries, and all the thoracic contents and cervical tissues up to the level of the thyroid gland were isolated postvertebrally as described by Toda & West (1967). The atria and attached sympathetic nerves were then set up in warm oxygenated nutrient solution (Toda & Shimamoto, 1968). In addition, conventional atrial preparations were made and used in the experiments in which the effects of noradrenaline and tyramine were investigated. The nutrient solution contained in 1,000 ml. of distilled water: sodium 162.1 mmoles, potassium 5.5 mmoles, calcium 2.2 mmoles, chlorine 157.0 mmoles, bicarbonate 14.9 mmoles, and dextrose 5.6 mmoles. The isolated specimens were fixed horizontally between hooks under a resting tension from 300 to 500 mg in a muscle bath of 60 ml. capacity. Hooks fixing the right atrium were connected to a force displacement transducer (Nihonkoden Kogyo Co.). The nutrient solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide and was maintained at $30° \pm 0.5°$ C. After mounting the preparation, 60–90 min were allowed for equilibration before measurements were begun.

One sympathetic nerve was lifted above the surface of the solution and was placed on a bipolar silver electrode. The sympathetic nerve was stimulated for 3 sec by a train of rectangular pulses of 1.0 msec duration at the frequency of either 5 or 20 pulses/sec with shocks of supramaximal intensity. The stimuli were provided by a Sanei type ES-103-Z pulse generator.

The drugs tested were applied directly to the muscle bath and cumulative dose-response curves were constructed. Only one series of curves for tyramine was obtained from any particular preparation. Preparations were exposed to desmethylimipramine, cocaine and procaine for 20 min before sympathetic stimulation, noradrenaline and tyramine were applied.

Drugs used in the present study were as follows: (±)-noradrenaline hydrochloride, tyramine hydrochloride, desmethylimipramine hydrochloride, (−)-cocaine hydrochloride and procaine hydrochloride. The final concentrations were expressed in terms of g/ml. of the salts.

The spontaneous rate and contractile force of the atria were recorded on a Sanei pen-writing oscilloscope. No attention was paid to the contractile force, because it is a function of atrial rate (Blinks & Koch-Weser, 1961) as well as the action of drugs. The atrial rate was estimated from the means of ten measurements of cycle length between contractions. All results shown in figures and the table are mean values ± standard errors. The significance of the differences between means was calculated by Student's *t* test.

RESULTS

Desmethylimipramine

Stimulation of sympathetic postganglionic fibres produced an increase in the atrial rate and the contractile force which depended on the frequency of stimulation. The maximum increase was attained 15–30 sec after the stimulation had been terminated. The time course of the positive chronotropic response to sympathetic stimulation at a frequency of 20 pulses/sec with and without desmethylimipramine is illustrated in Fig. 1. Values in the figure represent means of measurements obtained from eleven to fifteen preparations. According to Iversen (1965) 1.3×10^{-8}M desmethylimipramine produces 50% inhibition of the noradrenaline uptake by the isolated rat heart. The spontaneous rate was not significantly affected by desmethylimipramine 10^{-7} or 10^{-6} g/ml. The positive chronotropic response to sympathetic nerve stimulation was significantly potentiated in the presence of 10^{-7} g/ml. and after 10^{-6} g/ml. had been replaced with fresh nutrient solution. However, the response was only slightly potentiated by 10^{-6} g/ml. The maximum increase in the atrial rate was attained 30 to 60 sec after the termination of sympathetic stimulation in the presence of desmethylimipramine. Fig. 2 shows how the compound

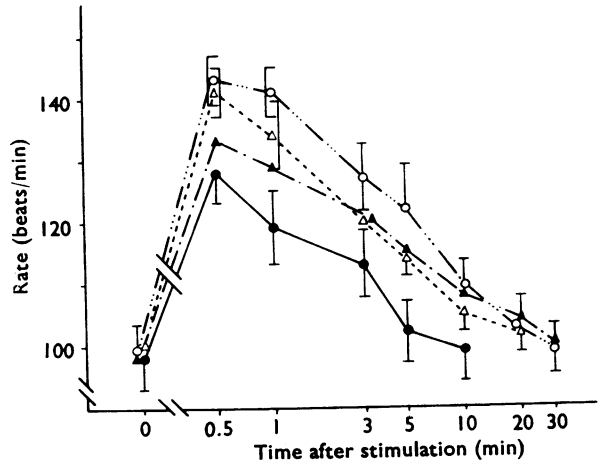

Fig. 1. Time course of the positive chronotropic effect of nerve stimulation at a frequency of 20 pulses/sec with and without desmethylimipramine. ●——●, Control; ▲---▲, desmethylimipramine 10^{-7} g/ml.; ▲—·—▲, desmethylimipramine 10^{-6} g/ml.; ○—···—○, results obtained 30–60 min after the preparation was treated with desmethylimipramine and washed.

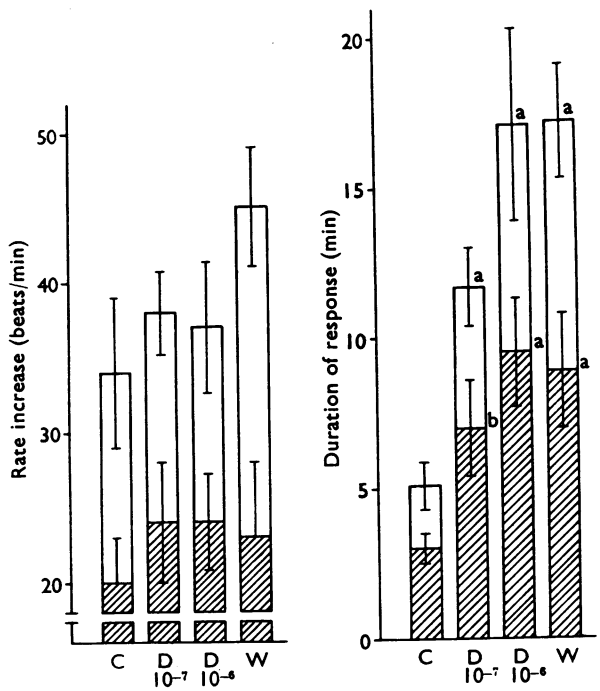

Fig. 2. Effects of desmethylimipramine on the maximum increase in the atrial rate and on the duration of the increase in response to sympathetic stimulation at frequencies of 5 (hatched columns) and 20 (open columns) pulses/sec. C, Control; D, desmethylimipramine; W, effects observed after repeated washing of the preparation. Vertical bars represent standard errors of the means. a: significant difference from control, $P<0.01$. b: $P<0.02$.

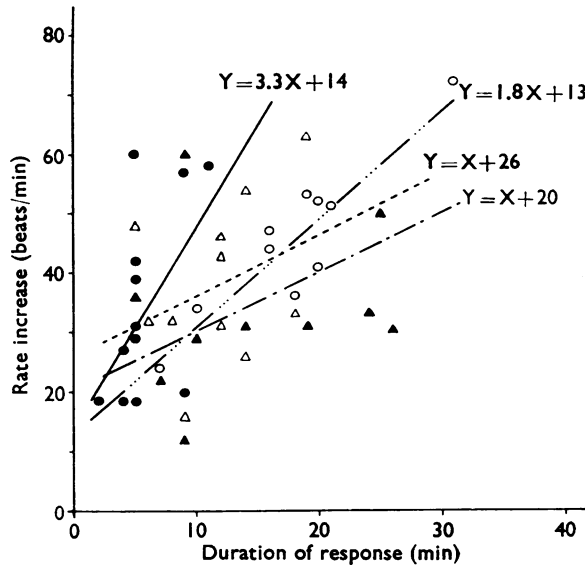

Fig. 3. Relationship between the rate increase and the duration of the increase in response to nerve stimulation at 20 pulses/sec. Equations in the figure were obtained by the method of least squares. ● —— ●, Control; ▲ - - - ▲, desmethylimipramine 10^{-7} g/ml.; ▲ —·— ▲ desmethylimipramine 10^{-6} g/ml.; ○ —···— ○, effects observed after repeated washing of the preparation.

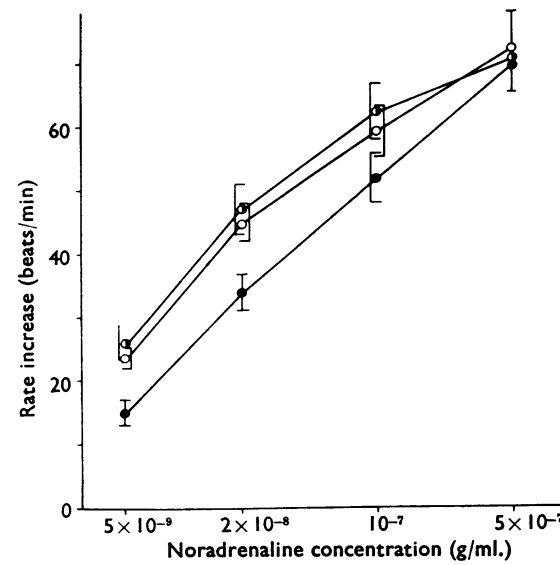

Fig. 4. Modifications by desmethylimipramine of the positive chronotropic effect of noradrenaline. The results are the means of fourteen to seventeen experiments. Mean values of the rate increase in response to two lower concentrations of noradrenaline were significantly different from the corresponding controls ($P<0.01$). ●——●, Control; ◑——◑, desmethylimipramine 10^{-7} g/ml.; ○——○, desmethylimipramine 10^{-6} g/ml.

affected the increase in the rate and the duration of the increase in response to sympathetic stimulation. A slight potentiation of the maximum increase in the atrial rate was produced, whereas the duration of the response was consistently prolonged in proportion to the concentration applied. The prolongation of the response was not reversed by repeated washing of the preparation. Usually the bigger the increase in the rate produced by sympathetic stimulation, the longer was the duration of the response. Fig. 3 illustrates the relationship and the slope of the regression lines calculated by the method of least squares. Desmethylimipramine markedly decreased the slope without reducing the maximum increase in the rate.

The positive chronotropic response to the application of noradrenaline in concentrations lower than 10^{-7} g/ml. was potentiated by desmethylimipramine, but there was apparently no potentiation of the effects of higher concentrations of the drug (Fig. 4).

The concentration-response curve for tyramine is shown in Fig. 5. The positive chronotropic response was significantly inhibited by desmethylimipramine 10^{-7} and 10^{-6} g/ml.

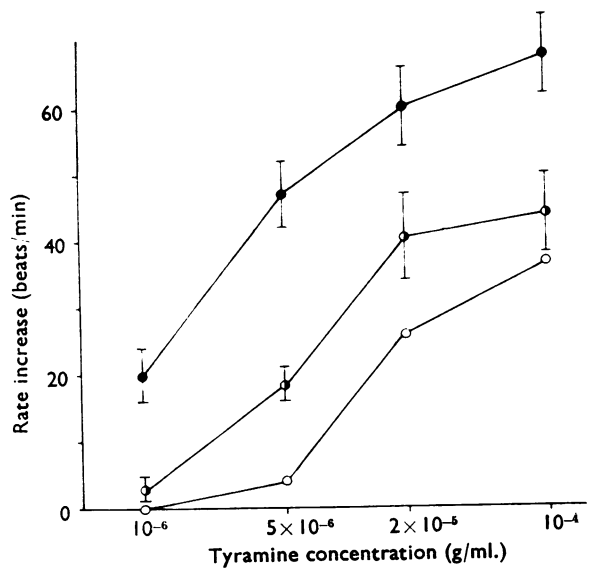

Fig. 5. Inhibition by desmethylimipramine of tachycardia induced by tyramine. The control results (●——●) are the mean of ten experiments, those with desmethylimipramine 10^{-7} g/ml. (◑——◑), of eight experiments, and those with 10^{-6} g/ml. (○——○), of three experiments. Values in the presence of desmethylimipramine were significantly less than the corresponding controls.

Cocaine

Iversen (1965) reported that 3.8×10^{-7}M cocaine produced 50% inhibition of the uptake of noradrenaline by the isolated rat heart. The atrial rate was not affected by 10^{-6} g/ml. but was significantly slowed by cocaine 10^{-5} g/ml. (from 91 ± 4 beats/min to 81 ± 2 beats/min, mean values for ten experiments). The effects of cocaine on the positive

TABLE 1

EFFECTS OF COCAINE ON THE POSITIVE CHRONOTROPIC RESPONSE TO SYMPATHETIC NERVE STIMULATION

* Significant difference from control, $P<0.01$. The figures show the increase in the rate and the duration of the increase in response to sympathetic nerve stimulation, at the frequency indicated, for 3 sec. The column headed "Total" shows the mean values for all the results, that headed "Potentiation" the mean values for five preparations in which the effects were increased, that headed "Inhibition" for three preparations in which the effects were reduced and that headed "No change" for two preparations in which the effects were not significantly different from the controls. The results marked "After wash" were obtained 30–40 min after the preparation had been treated with cocaine 10^{-5} g/ml. and washed repeatedly.

Stim. freq.	Procedure	Total			Potentiation			Inhibition			No change		
		N	Rate increase (beats/min)	Duration (min)	*N*	Rate increase (beats/min)	Duration (min)	*N*	Rate increase (beats/min)	Duration (min)	*N*	Rate increase (beats/min)	Duration (min)
	Control	10	31·6±3·1	5·2±0·6									
20/sec	Coc. 10^{-6}	10	37·5±5·3	6·3±1·0	5	52·6*±4·8	8·0 ±1·4	3	16·7	4·3	2	31·0	7·0
	Coc. 10^{-5}	10	33·7±7·5	12·0±2·2	5	53·4*±6·2	17·0*±2·8	4	12·3*±3·4	6·0±1·0	1	29·0	9·0
	After wash	10	39·0±5·4	9·7±2·3	5	49·8*±6·0	12·2*±3·0	4	33·0 ±5·7	7·0±0·8	1	31·0	8·0
	Control	7	13·3±2·4	2·4±0·4									
5/sec	Coc. 10^{-6}	7	13·6±3·4	3·3±0·8	3	19·7	4·3	2	6·0	2·5	2	12·0	2·5
	Coc. 10^{-5}	7	16·4±4·6	4·4±0·9	2	29·5	6·5	3	5·0	2·3	2	15·5	5·5
	After wash	7	17·2±3·3	3·5±0·9	2	25·0	5·5	3	15·3	2·3	2	14·0	3·0

chronotropic response to sympathetic stimulation (at 20 pulses/sec) were variable. In five preparations the response was potentiated, in three it was inhibited and in two it was unchanged. The results are summarized in Table 1. When cocaine potentiated the response, it was observed that its effects persisted after repeated washing of the preparation, whereas when the response was inhibited by cocaine its effects were easily reversed by washing.

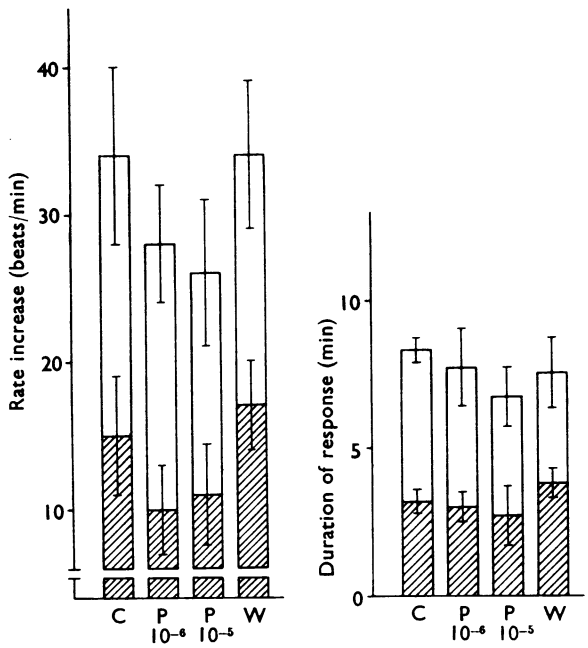

Fig. 6. Effects of procaine on the rate increase and on the duration of the increase in response to sympathetic stimulation at frequencies of 5 (hatched columns) and 20 (open columns) pulses/sec. Each column is the mean of six experiments. C, Control; P, procaine; W, effects observed after repeated washing of the preparation.

Procaine

Procaine is known to be slightly less effective than cocaine in producing a conduction block of the isolated sciatic nerve of frog (Bennett, Wagner & McIntyre, 1942). The atrial rate was not affected by either 10^{-6} or 10^{-5} g/ml. of procaine but slightly slowed by 5×10^{-5} g/ml. The positive chronotropic effect of sympathetic stimulation was slightly but consistently inhibited by procaine in concentrations ranging from 10^{-6} to 5×10^{-5} g/ml., whereas the duration of the effect was not altered. The effects of procaine on the preparation were easily reversed by washing (Fig. 6).

DISCUSSION

It is known that desmethylimipramine greatly reduces or completely abolishes the pressor actions of tyramine, amphetamine and guanethidine, and reverses the actions of bretylium in anaesthetized and spinal cats (Cuenca, Salva & Valdecasas, 1964), whereas

it potentiates pressor actions of noradrenaline (Sigg *et al.*, 1963). A similar reduction of the positive chronotropic effect of tyramine and potentiation of the effect of noradrenaline was observed in isolated rabbit atria in the present study. Desmethylimipramine produced a marked prolongation of the atrial responses to sympathetic nerve stimulation but only a slight potentiation of the maximum increase in the atrial rate. Recent studies indicate that many drugs, including desmethylimipramine and cocaine, which inhibit the uptake of noradrenaline by sympathetic nerve terminals, potentiate the action of noradrenaline because the uptake is one of the physiologically important mechanisms by which the effects of the adrenergic transmitter are terminated (Axelrod, Weil-Malherbe & Tomichik, 1959 ; Whitby, Axelrod & Weil-Malherbe, 1961). If desmethylimipramine interferes with the uptake of noradrenaline by the storage sites of sympathetic nerves, higher concentrations of the amine will be available to activate adrenaline receptors (Furchgott *et al.*, 1963 ; Trendelenburg, 1965). The uptake mechanism of this kind, however, seems to contribute more effectively to prolonging the actions of cardiac noradrenaline than to increasing the size of the response, so the prolongation should be correlated with an inhibition of the removal of noradrenaline and re-uptake by sympathetic nerve terminals.

On the other hand, a reduction of the responses to indirectly acting sympathomimetic amines, such as tyramine and amphetamine, seems to be caused by an antagonism of the uptake of the amines by sympathetic nerve terminals (Trendelenburg, 1961). Evidence that the positive chronotropic effect of sympathetic nerve stimulation was not reduced but potentiated by desmethylimipramine supports Trendelenburg's hypothesis, and rules out the possibility that it interferes with the electrical and chemical release of cardiac noradrenaline (Fleckenstein & Stöckle, 1955 ; Burn & Rand, 1958). Although the autonomic and sedative actions of reserpine are antagonized by prior treatment of animals with desmethylimipramine, the depletion of brain amines induced by reserpine is not affected (Costa, Garattini & Valzelli, 1960 ; Garattini, Giachetti, Jori, Pieri & Valzelli, 1962). Rises in blood pressure produced by α-phenyl-α-ethylacetylcholine iodine, a nicotine-like ganglionic stimulant, and by acetylcholine, are, however, reduced by desmethylimipramine (Cuenca *et al.*, 1964). If the latter does not interfere with the release of cardiac noradrenaline induced by the ganglionic stimulants, it seems possible that it blocks the action of the stimulants at receptor sites in the sympathetic ganglia and adrenal medulla.

In anaesthetized, vagotomized dogs different workers have observed different effects with cocaine. Moore (1966) found that it markedly potentiated the cardiac and pressor responses to sympathetic nerve stimulation, whereas Pappas, Margolius & Gaffney (1965) observed no potentiation of cardiac sympathetic effects although the effects of added noradrenaline were potentiated. It is reported that in isolated rabbit atria the positive chronotropic responses to sympathetic stimulation are potentiated by cocaine and phenoxybenzamine (Huković, 1959). In the present study the effects of cocaine were varied ; in some preparations a potentiation was observed of the effects of sympathetic stimulation and this potentiation was not reversed by repeated washing of the preparation. In other preparations the effects of sympathetic stimulation were inhibited, as they were also by procaine, and these effects were easily reversed by washing the preparation. It is suggested that this inhibitory action of cocaine is a result of its local anaesthetic properties, whereas the potentiation is a result of an action like that of desmethylimipramine.

SUMMARY

1. Sympathetic nerve-atrial preparations and conventional atrial preparations isolated from rabbits were used. Stimulation of either right or left sympathetic nerve for 3 sec at a frequency of 20 pulses/sec induced a marked acceleration of the atrial rate and an increase in the contractile force which persisted for 3–5 min.

2. The positive chronotropic response to nerve stimulation was slightly potentiated by desmethylimipramine 10^{-7} and 10^{-6} g/ml., and the duration of the response was markedly prolonged. These effects were not reversed by repeated washing of the preparation.

3. The application of desmethylimipramine shifted the concentration-response curve of noradrenaline to the left but the concentration-response curve of tyramine to the right.

4. Cocaine potentiated the positive chronotropic effect of sympathetic nerve stimulation in five of the atria studied, inhibited it in three and in two preparations there was no change in response. The positive chronotropic effect was slightly but consistently inhibited by procaine and the inhibition was reversed by repeated washing.

5. These results suggest that the potentiation by desmethylimipramine of the positive effects of sympathetic stimulation and of noradrenaline, and the inhibition by desmethylimipramine of the effects of tyramine, result from an inhibition of the uptake of endogenous and exogenous noradrenaline and tyramine by sympathetic nerve terminals on the effector organ, and that cocaine possesses a similar potentiating action and also a procaine-like inhibitory action on the positive chronotropic response to sympathetic stimulation.

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