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Interactions of inhibitors of noradrenaline uptake and angiotensin on the sympathetic nerves of the isolated rabbit heart

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Summary

1. The interaction of angiotensin and several inhibitors of the uptake of noradrenaline across the neuronal membrane (cocaine, desipramine, protriptyline, and pronethalol) on the output of noradrenaline produced by sympathetic nerve stimulation has been studied in the isolated perfused rabbit heart.

2. Most of these drugs increased noradrenaline outflow—angiotensin, for example, by 175%. Cocaine (10^{-4}M) did not change the amine overflow, probably because this very high concentration inhibited not only the re-uptake but also the liberation of noradrenaline.

3. Desipramine, protriptyline, and pronethalol, although infused in concentrations which enhanced the noradrenaline output, were not able to impair the angiotensin-induced increase of transmitter overflow. In the presence of cocaine (10^{-4}M) the increase elicited by angiotensin was slightly reduced, though lower concentrations of cocaine, as previously described, do not interfere with the effect of angiotensin.

4. In contrast to the interaction between uptake inhibitors and angiotensin, the augmented output of noradrenaline caused by an uptake inhibitor could not be increased further by infusion of a second uptake inhibitor.

5. It is concluded that the increase of the outflow of noradrenaline during sympathetic nerve stimulation by small doses of angiotensin is not caused by an inhibition of re-uptake. On the contrary, the transmitter liberation seems to be facilitated. This is a novel principle of drug action on the sympathetic nerve terminals.

Introduction

Angiotensin increases the output of noradrenaline from isolated rabbit hearts caused by stimulation of the sympathetic cardiac nerves (Starke, Werner & Schümann, 1969). This effect is not due to coronary constriction, for infusion of vasopressin or diminution of the delivery of the perfusion pump decrease, rather than augment, noradrenaline overflow (Starke, Werner, Hellerforth & Schümann, 1970).

Noradrenaline released from sympathetic nerve terminals is mainly removed from the extracellular space by re-uptake (Folkow, Häggendal & Lisander, 1967). A blockade of this pathway of inactivation is one way of increasing transmitter

overflow. In high concentrations (10^{-5}M) angiotensin inhibits noradrenaline uptake (Schümann, Starke, Werner & Hellerforth, 1970), whereas the concentrations required to augment the output of noradrenaline evoked by sympathetic nerve stimulation are much lower (10^{-10}M ; Starke *et al.*, 1969).

In order to obtain further information about the mechanisms involved, we tested the interactions, on the output of noradrenaline, of angiotensin and some typical inhibitors of amine uptake across the nerve membrane. Persistence of the angiotensin-induced increase of noradrenaline output despite previous blockade of reuptake by a second agent would favour the assumption of a different mechanism of action of the peptide.

Methods

Noradrenaline output during cardiac nerve stimulation

Isolated rabbit hearts with intact sympathetic nerve supply were prepared and perfused with modified Tyrode solution at a constant rate of 25 ml/min as previously described (Starke *et al.*, 1969). Contractions were recorded on a smoked drum by means of an isotonic lever. The sympathetic nerves were stimulated for periods of 1 min (Stimulator II, H. Sachs, Hugstetten ; 5 Hz, 3 ms duration, 8 mA). During these periods the nerves of the right and left side were stimulated alternately, each side twice for 15 s. Four stimulation periods (S_1–S_4) separated by intervals of 14 min were applied in each experiment. To determine the output of noradrenaline, 2 min samples of the venous effluent were collected, starting with the onset of the electrical stimulation. The resting output was estimated in 2 min samples collected in the intervals. Drugs were infused into the aortic cannula from 8 min before S_2 and/or 8 min before S_4 until the end of the experiment.

Removal of noradrenaline from the perfusion medium

Isolated rabbit hearts were perfused with modified Tyrode solution at a constant rate of 25 ml/min. After 1 h of equilibration, (−)-noradrenaline was infused into the aortic cannula to give a final concentration of 10 ng/ml. Four 50 ml samples of the perfusate were successively collected, starting 2 min after the onset of the infusion. The amount of noradrenaline removed during passage through the coronary vessels was averaged for the four samples and calculated as percentage of the amount infused. Drugs tested for their influence on amine removal were infused 10 min before and during noradrenaline infusion.

Noradrenaline determination

The catechols were adsorbed on Al_2O_3 (Aluminiumoxid basisch, Woelm, Eschwege), eluted with 0·1 N HCl, and determined fluorimetrically (von Euler & Floding, 1955 ; Palmer, 1964). Values are corrected for recovery from Al_2O_3. The output of adrenaline from isolated rabbit hearts is negligible (Huković & Muscholl, 1962).

Drugs

A final concentration in the perfusion fluid of $1{\cdot}2 \times 10^{-9}$M angiotensin (Val^5-angiotensin II-Asp^1-β-amide ; hypertensin, Ciba, Basel) was used in all experiments.

Cocaine hydrochloride (Merck, Darmstadt); desipramine hydrochloride (Geigy, Basel); protriptyline hydrochloride (Merck, Sharp & Dohme, Rahway, N.J.); (±)-pronethalol hydrochloride (Rhein-Pharma, Heidelberg); (−)-noradrenaline base (Höchst, Frankfurt/M.). The drugs did not interfere with the purification and fluorimetric assay of noradrenaline.

Means ± S.E. are given throughout this paper. Student's t test was used to calculate significance.

Results

The resting output of noradrenaline (0–5 ng/2 min) was not significantly changed by any of the drugs or drug combinations tested. In control experiments, the outflow of noradrenaline elicited by sympathetic nerve stimulation was highest during S_1 and decreased progressively during the subsequent stimulation periods; for each period the release amounted to about 90% of the preceding one (Table 1, group 1). Perfusion with $1{\cdot}2 \times 10^{-9}$M angiotensin during S_4, however, augmented noradrenaline overflow to 175% of S_3 (Table 1, group 2; $P<0{\cdot}001$). This value agrees well with our previous results (Starke *et al.*, 1969).

Desipramine, protriptyline, and angiotensin

The influence of desipramine and protriptyline on the angiotensin-induced increase of noradrenaline output is illustrated in Fig. 1. Protriptyline and desipramine were infused from 8 min before S_2 until the end of the experiment. Both drugs augmented the overflow of noradrenaline; overflow was maximal during S_2 and declined during the following stimulation periods. If, however, angiotensin was added before and during S_4, the amine output caused by S_4 was further increased. For comparison with the effect of angiotensin alone, amine output during S_4 was calculated as a percentage of that during S_3. In the presence of desipramine or protriptyline, angiotensin increased the overflow of noradrenaline during S_4 to 168 ± 10% or 164 ± 9% of S_3, respectively. There is no significant difference between

TABLE 1. *Interaction of angiotensin, cocaine and pronethalol on the output of noradrenaline from isolated rabbit hearts during sympathetic nerve stimulation*

	Drug (concentration in the perfusion medium)	Noradrenaline output in % of the preceding stimulation period				
		S_1	S_2	S_3	S_4	N
(1)	None		97·8 ± 4·8	84·0 ± 7·1	88·4 ± 3·8	7
		43·4 ± 7·2	*42·3 ± 7·4*	*36·1 ± 7·6*	*32·5 ± 7·3*	
(2)	Angiotensin $1{\cdot}2 \times 10^{-9}$M		85·7 ± 4·7	88·2 ± 4·2	174·5 ± 16·9	5
		37·0 ± 4·8	*31·8 ± 4·9*	*28·5 ± 5·1*	*50·9 ± 10·8*	
(3)	Cocaine 10^{-4}M Angiotensin $1{\cdot}2 \times 10^{-9}$M		88·7 ± 6·9	88·3 ± 3·0	126·0 ± 15·7	8
		44·1 ± 4·7	*39·2 ± 4·6*	*34·6 ± 4·1*	*45·0 ± 7·5*	
(4)	Pronethalol 10^{-5}M		189·8 ± 14·5	87·2 ± 3·3	86·6 ± 5·6	5
		50·6 ± 9·2	*95·7 ± 16·0*	*85·1 ± 15·9*	*70·9 ± 11·5*	
(5)	Pronethalol 10^{-5}M Angiotensin $1{\cdot}2 \times 10^{-9}$M		161·1 ± 5·5	84·7 ± 5·1	174·9 ± 13·2	7
		53·6 ± 11·9	*86·2 ± 19·8*	*67·8 ± 12·7*	*114·8 ± 22·5*	
(6)	Pronethalol 5×10^{-5}M Angiotensin $1{\cdot}2 \times 10^{-9}$M		147·4 ± 17·0	80·7 ± 7·4	164·7 ± 12·7	8
		38·5 ± 6·1	*51·0 ± 5·9*	*42·6 ± 6·9*	*72·3 ± 14·5*	

Figures in italics: absolute values in ng.
The sympathetic nerves were stimulated for 1 min periods (S_1–S_4) separated by 14 min intervals. The uptake inhibitors were infused into the aortic cannula from 8 min before S_2, angiotensin from 8 min before S_4 until the end of the experiment. Means ± S.E.

the effect of angiotensin in presence and in absence (175%, see above) of desipramine and protriptyline.

A typical experiment is shown in Fig. 2a. Protriptyline augments the effects of sympathetic nerve stimulation on noradrenaline output and heart rate ; the positive inotropic effect is prolonged. The amine output is further augmented by angiotensin, the positive inotropic effect is slightly increased, while the chronotropic effect is unchanged.

Pronethalol and angiotensin

The interaction of angiotensin and pronethalol on the output of noradrenaline is summarized in Table 1. Pronethalol (10^{-5}M) increased the outflow of noradrenaline to $177 \pm 7\%$ of the preceding stimulation period ($N=16$; all experimental groups combined). If the infusion was continued, amine output remained elevated during the subsequent stimulation periods, except for a decline comparable to that of control hearts (Table 1, group 4). If, however, angiotensin was added before and during S_4, amine output was further augmented to 175% of S_3 (Table 1, group 5 ; $P<0{\cdot}001$, compared with group 4).

Pronethalol (5×10^{-5}M) increased the output of noradrenaline caused by S_2 to 147% of S_1 (Table 1, group 6). This increase is smaller than that seen with 10^{-5}M pronethalol ($0{\cdot}05<P<0{\cdot}1$). Angiotensin, infused before and during S_4, caused a further augmentation, S_4 amounting to 165% of S_3. The angiotensin-induced increase of noradrenaline output is thus not antagonized by previous treatment with pronethalol.

A typical experiment is shown in Fig. 2b. Pronethalol reduces the amplitude of contraction. The positive chronotropic effect of sympathetic nerve stimulation is

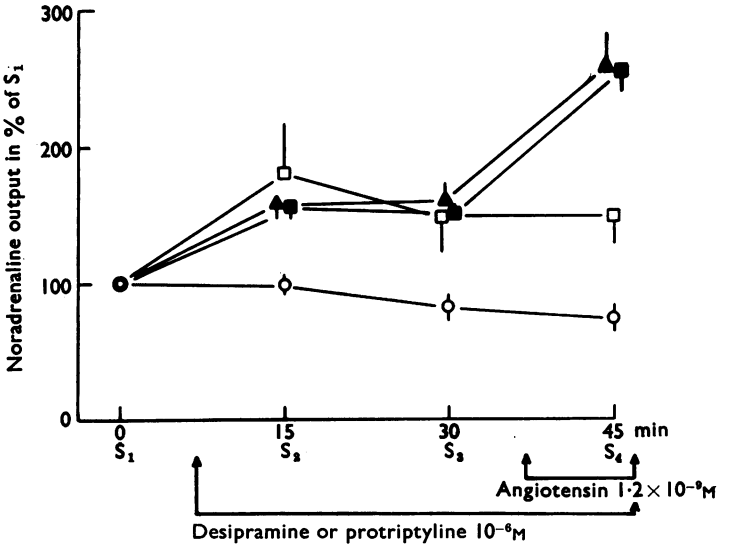

FIG. 1. Interaction of angiotensin, desipramine and protriptyline on the output of noradrenaline from isolated rabbit hearts caused by sympathetic nerve stimulation. ▲, Protriptyline + angiotensin ($N=5$) ; ■, desipramine + angiotensin ($N=7$) ; □, desipramine ($N=3$) ; ○, controls ($N=7$). Four 1 min stimulation periods (S_1–S_4) were applied in each experiment. The output of noradrenaline was calculated as percentage of S for each stimulation period. Means ± S.E.

abolished, the inotropic effect is greatly reduced. Angiotensin distinctly augments the amplitude of contraction. Despite the block of nearly all of the functional consequences of sympathetic nerve stimulation, the outflow of noradrenaline is increased by pronethalol and, further, by angiotensin.

Cocaine and angiotensin

During perfusion with 10^{-4}M cocaine from 8 min before S_2 onward, the output of noradrenaline evoked by S_2 was only about as high as would be expected in control

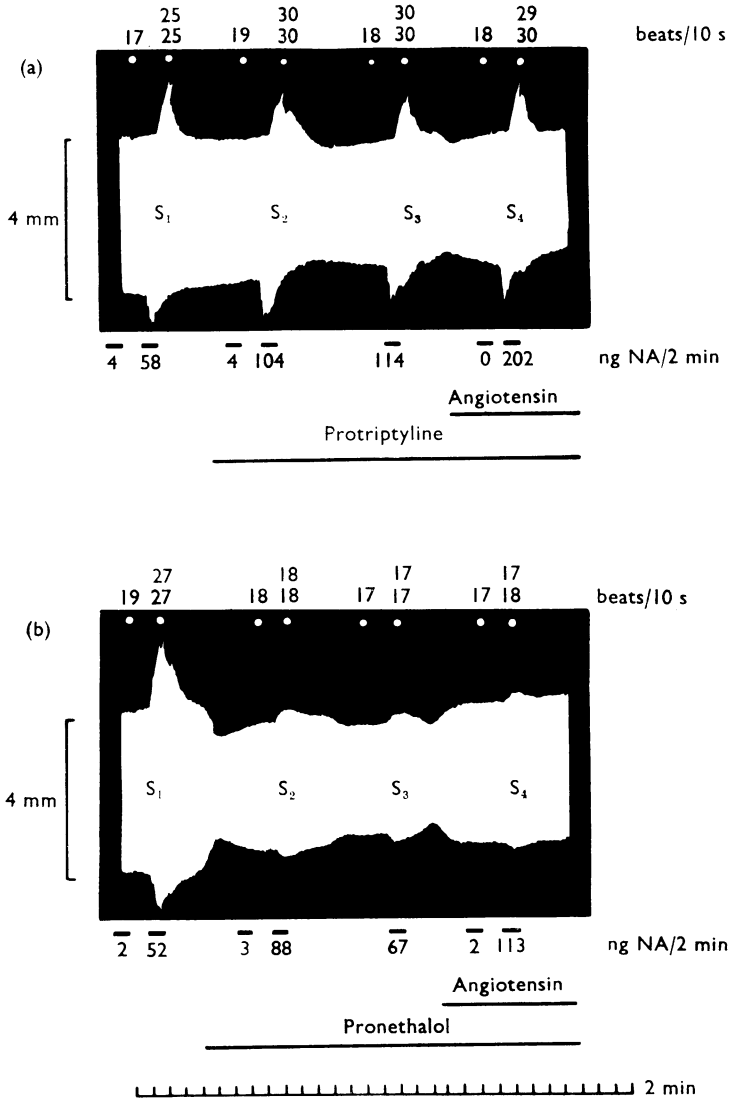

FIG. 2. Interaction of protriptyline 10^{-6}M (a) and pronethalol 10^{-5}M (b) with angiotensin $1{\cdot}2 \times 10^{-9}$M on the isolated perfused rabbit heart. Four 1 min periods of electrical stimulation of the sympathetic cardiac nerves were applied in each experiment (S_1–S_4). During stimulation, the heart rate was counted twice, 20–30 and 35–45 s after the onset of stimulation. Below the mechanograms the 2 min periods of perfusate collection and the noradrenaline content of the perfusates are indicated.

conditions (Table 1, group 3). Lower concentrations of cocaine have been shown to enhance amine overflow (Starke, 1970). If angiotensin was infused additionally 8 min before and during S_4, amine output was augmented to 126% of S_3—that is, less than in the absence of cocaine ($0{\cdot}05<P<0{\cdot}1$, compared with group 2). By this very high concentration of cocaine, the enhancing effect of angiotensin is partly, though not significantly, antagonized.

Combinations of several inhibitors of noradrenaline uptake

In these experiments, the interactions of several inhibitors of noradrenaline uptake on the output of transmitter caused by sympathetic nerve stimulation were examined (Table 2). Desipramine 10^{-6}M, cocaine 5×10^{-5}M or pronethalol 10^{-5}M were infused from 8 min before S_2 onward, and cocaine $1{\cdot}7\times10^{-5}$M or 5×10^{-5}M or desipramine 10^{-6}M were given, in addition, from 8 min before S_4 until the end of the experiment. While the first agent always caused the expected increase of noradrenaline output, the second one was, in every case, without any further enhancing effect.

Effects of desipramine, protriptyline, and pronethalol on noradrenaline uptake

We investigated the influence of desipramine, protriptyline and pronethalol on the removal of infused noradrenaline (final concentration 10 ng/ml) from the medium perfusing the hearts. Removal is chiefly a consequence of net-uptake across the neuronal membrane (Lindmar & Muscholl, 1964). Results are presented in Table 3. The removal of noradrenaline was almost completely prevented by all three drugs.

TABLE 2. *Interaction of some amine uptake inhibitors on the output of noradrenaline from isolated rabbit hearts during sympathetic nerve stimulation*

	Drug (concentration in the perfusion medium)	Noradrenaline output in % of the preceding stimulation period				
		S_1	S_2	S_3	S_4	N
(1)	Desipramine 10^{-6}M		139·0±12·9	88·5±4·9	89·4±9·9	4
	Cocaine $1{\cdot}7\times10^{-5}$M	*63·5±13·5*	*85·1±15·1*	*77·3±15·9*	*71·0±17·5*	
(2)	Desipramine 10^{-6}M		155·8±11·9	81·0±7·4	77·6±8·3	3
	Cocaine 5×10^{-5}M	*53·0±10·0*	*80·9±12·4*	*66·9±14·3*	*53·8±15·5*	
(3)	Cocaine 5×10^{-5}M		155·7±16·5	91·0±3·7	87·8±7·3	3
	Desipramine 10^{-6}M	*44·8±4·0*	*68·6±4·3*	*62·5±5·0*	*54·2±0·3*	
(4)	Pronethalol 10^{-5}M		188·4±18·0	86·2±1·9	82·9±6·3	4
	Cocaine $1{\cdot}7\times10^{-5}$M	*65·5±9·1*	*120·1±11·2*	*103·4±10·0*	*84·8±8·1*	

Figures in italics: absolute values in ng.
The sympathetic nerves were stimulated for 1 min periods (S_1–S_4) separated by 14 min intervals. The first drug of each group was infused into the aortic cannula from 8 min before S_2, the second one from 8 min before S_4 until the end of the experiment. Means±S.E.

TABLE 3. *Influence of some drugs on the removal of noradrenaline from the fluid perfusing isolated rabbit hearts*

Drug	Concentration (M)	Percentage of noradrenaline removed±S.E.	N
—	—	41·1±1·3	12
Desipramine	10^{-6}	2·8±2·6*	7
Protriptyline	10^{-6}	6·2±2·5*	4
Pronethalol	5×10^{-5}	4·1±6·6*	3

(−)-Noradrenaline (final concentration 10 ng/ml) was infused into the aortic cannula. The noradrenaline content of 50 ml samples of the venous effluent was determined, and the amount removed by the heart was calculated as percentage of the amount infused. Infusions of uptake inhibitors started 10 min before noradrenaline infusions. Means±S.E.
* Significantly different from controls ($P<0{\cdot}0001$).

Discussion

Our experiments demonstrate that, in the isolated rabbit heart, the uptake of exogenous noradrenaline (10 ng/ml) across the sympathetic nerve membranes is largely prevented by pronethalol, desipramine and protriptyline (see Lindmar & Muscholl, 1964 ; Carlsson & Waldeck, 1965 ; Iversen, 1965). The same has been shown for cocaine (Lindmar & Muscholl, 1964). By inhibiting the re-uptake of noradrenaline liberated during sympathetic nerve stimulation, all these drugs increase noradrenaline overflow into the venous effluent. Cocaine, however, though augmenting amine overflow at concentrations of $1{\cdot}7 \times 10^{-5}$M and 5×10^{-5}M (Starke, 1970), failed to do so at 10^{-4}M. Similarly, 5×10^{-5}M pronethalol enhanced amine output less than 10^{-5}M. It is very likely, therefore, that the higher concentrations of these agents inhibit noradrenaline release as well as re-uptake (Huković & Muscholl, 1962 ; Malmfors, 1969).

Prevention of the uptake of small amounts of exogenous noradrenaline does not imply a similarly effective inhibition of re-uptake at the high concentrations occurring after endogenous transmitter release. We therefore examined the interactions of several inhibitors of amine uptake on the output of noradrenaline caused by sympathetic nerve stimulation. During perfusion with cocaine, desipramine, or pronethalol, an additional infusion of a second uptake inhibitor did not further increase noradrenaline overflow. In the doses used, the block caused by the first uptake inhibitor seems to be too effective to be significantly enhanced by a second one.

In contrast to these drugs, angiotensin augments the outflow of noradrenaline beyond the level reached by previous inhibition of re-uptake. Our first experiments (Starke, 1970) were performed with cocaine as a typical blocking agent. In order to exclude any specific interaction between angiotensin and an individual drug, a variety of agents were used in the present series which, except for their ability to interfere with amine transport, have quite different cardiac effects (Fig. 2). If the peptide alone was infused during S_4, the output of noradrenaline was increased to 175% of S_3. If 10^{-6}M desipramine, 10^{-6}M protriptyline, or 10^{-5}M or 5×10^{-5}M pronethalol were present from S_2 until the end of the experiment, the increase caused by angiotensin, infused additionally during S_4, amounted to 168, 163, 175 or 165% of S_3, respectively. These values are in good agreement with those reported, under equal conditions, for $1{\cdot}7 \times 10^{-5}$M or 5×10^{-5}M cocaine (181 and 171%, respectively ; Starke, 1970). The action of angiotensin on noradrenaline output is thus virtually unaffected by the presence of a block of re-uptake, irrespective of the nature of the blocking agent.

If angiotensin enhanced the output of noradrenaline by interference with re-uptake, its efficacy should be reduced in presence of a partial previous uptake inhibition by another drug; it should be abolished in presence of a complete previous block of uptake. Our results favour the assumption of a mechanism of action of angiotensin independent of noradrenaline re-uptake.

This postulate is supported by the fact that much higher doses of angiotensin are needed to diminish the removal of noradrenaline from the perfusion medium than to increase amine outflow (Schümann, Starke, Werner & Hellerforth, 1970). Moreover, whereas amine uptake inhibitors impede the release of noradrenaline by tyramine, angiotensin fails to do so (unpublished results in isolated rabbit hearts).

Similar conclusions have been reached by Zimmerman & Gisslen (1968), Day & Owen (1969) and Hughes & Roth (1969).

Re-uptake is the most important way of inactivation of noradrenaline released from the sympathetic nerve terminals. The major part of the remaining fraction escapes into the blood or perfusion medium, enzymatic degradation being largely negligible (Folkow, Häggendal & Lisander, 1967). Taking this concept for granted, the conclusion is justified that angiotensin increases the amount of noradrenaline released during sympathetic nerve stimulation rather than impeding re-uptake. In the absence of sympathetic nerve discharges angiotensin does not significantly influence the output of noradrenaline. This mechanism seems to be a novel principle of drug action on the sympathetic nerve terminals.

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