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The influence of angiotensin on the uptake of noradrenaline by the isolated heart of the rabbit

H. J. SCHÜMANN, K. STARKE, U. WERNER AND R. HELLERFORTH

Institute of Pharmacology, Ruhr-University, Essen, Germany

The influence of angiotensin on the removal of noradrenaline (10 ng/ml) from the perfusion medium and on the net-uptake of noradrenaline (5 and 20 μg/ml) was examined in the rabbit isolated heart. Concentrations of angiotensin, known to augment the output of noradrenaline from rabbit heart during sympathetic nerve stimulation, did not inhibit the removal of infused noradrenaline from the perfusion fluid. Only very high concentrations of angiotensin (13 μg/ml) significantly diminished the loss of noradrenaline. The positive inotropic effect of noradrenaline was not potentiated by angiotensin. On the other hand, cocaine, in doses which enhance the output of noradrenaline during sympathetic stimulation greatly reduced the loss of noradrenaline from the perfusion fluid. Neither angiotensin (130 ng/ml) nor metanephrine (5 and 20 μg/ml) significantly influenced the net-uptake of noradrenaline from high concentrations. The observations made support the assumption that an increase of transmitter liberation rather than inhibition of transmitter inactivation is responsible for the increase caused by angiotensin in the output of noradrenaline during stimulation of sympathetic nerves.

Noradrenaline is released into the perfusion medium during stimulation of the postganglionic sympathetic nerves of rabbit isolated hearts (Huković & Muscholl, 1962). This outflow of noradrenaline is augmented if angiotensin is infused into the aortic cannula (Starke, Werner & Schümann, 1969). A similar increase of amine output has been observed in the dog paw and kidney (Zimmerman & Whitmore, 1967; Zimmerman & Gisslen, 1968) but at higher concentrations of angiotensin.

Noradrenaline released from the sympathetic nerve terminals is largely inactivated by re-uptake through the neuronal membrane (Iversen, 1967). The same mechanism is mainly responsible for the removal of noradrenaline from fluid perfusing an isolated organ. Cocaine, which increases the output of noradrenaline during sympathetic stimulation by interference with re-uptake, also prevents removal of noradrenaline from the perfusion fluid (Huković & Muscholl, 1962; Lindmar & Muscholl, 1964). High concentrations of angiotensin diminish the uptake of [^{3}H]noradrenaline into several organs (e.g. Palaić & Khairallah, 1967a). If the peptide augments the output of noradrenaline induced by sympathetic nerve stimulation by inhibition of re-uptake, it should also reduce the amount of noradrenaline removed from the perfusion fluid.

We tested this possibility and examined additionally the influence of angiotensin on the net-uptake of noradrenaline into rabbit isolated hearts at high concentrations of noradrenaline.

EXPERIMENTAL

Rabbits of either sex (89), 1·5-2·0 kg, were killed by a blow on the head. The hearts were immediately removed and perfused at 34° or 37° and at a constant flow rate of 10 or 25 ml/min (roller pump Desaga, Heidelberg). By means of a four-way stopcock, several physiological salt solutions (PSS) could be perfused successively. Modified Tyrode solution (Starke & others, 1969) or Krebs solution (Peach, Bumpus & Khairallah, 1969) were used. Ascorbic acid and disodium-EDTA 10 mg/litre were always added, and the solution was saturated with a mixture of 5% carbon dioxide in oxygen. Contractions were monitored by means of a strain gauge connected to a Hellige multiscriptor, diastolic tension being adjusted to 2 g. Experiments were started after 45 or 120 min perfusion with normal PSS.

To estimate the removal of noradrenaline from the perfusion medium, the hearts were perfused with PSS containing 10 ng/ml (—)-noradrenaline. Four 4 min samples (if the perfusion rate was 10 ml/min) or 2 min samples (if the perfusion rate was 25 ml/min) of the venous effluent were successively collected, starting 10 s or 2 min after the onset of noradrenaline perfusion, respectively. The samples were acidified and analysed for noradrenaline.

The uptake of noradrenaline at high concentrations was measured in hearts perfused with Tyrode solution at 25 ml/min 125 or 500 μg/min (—)-noradrenaline were infused into the aortic cannula (infusion apparatus Unita, Braun, Melsungen), giving final concentrations of 5 or 20 μg/ml, respectively. Perfusion with 20 μg/ml was carried out for 10 min, followed by a 2 min washout, perfusion at 5 μg/ml for 5 min, without a final wash-out. The hearts were then removed from the apparatus, rinsed with PSS, blotted, weighed, and homogenized by means of an Ultra-Turrax (Janke + Kunkel, Staufen) in 20 ml 0·4N $HClO_4$ containing 0·1% EDTA and ascorbic acid. After centrifugation, the residue was once more extracted and the final volume made up to 50 ml. 10 ml aliquots were analysed for noradrenaline.

The catecholamines were adsorbed on Al_2O_3 (Aluminiumoxid basisch, Woelm, Eschwege) by stirring at pH 8·5. After elution with 0·1N HCl, noradrenaline was determined fluorimetrically as described by Euler & Floding (1956) and Palmer (1964). The recovery from alumina was tested for each series of samples adsorbed on one occasion by adding appropriate amounts of noradrenaline to perfusates or extracts. Values are corrected for the corresponding recovery (mean: 69·4 $\pm$ 0·8%; N = 54). Recovery of 40 μg noradrenaline added before homogenization of hearts was 85·0 $\pm$ 6·3%; N = 3).

Drugs. Val^5-angiotensin II-Asp^1-β-amide (Hypertensin, Ciba AG, Basel); (—)-noradrenaline base (Farbwerke Hoechst AG, Frankfurt/M.); cocaine hydrochloride (Merck AG, Darmstadt); (±)-metanephrine hydrochloride (Calbiochem AG, Luzern). Doses refer to the bases.

Mean values $\pm$ s.e. are given throughout. Student's *t*-test was used to calculate significance. n = number of experiments.

RESULTS

Noradrenaline elimination

Nearly equal amounts of noradrenaline were recovered from the four venous effluent samples collected in each experiment. The four values were averaged to calculate the percentage of noradrenaline removed during passage through the coron-

ary vessels and, further, the rate of elimination, expressed as ng noradrenaline/heart min^{-1} and ng noradrenaline/g heart min^{-1}.

Data are presented in Table 1. At a perfusion rate of 25 ml/min, the control hearts removed 41·1% of the perfused noradrenaline; 66·1% was removed at the lower

Table 1. *Effect of angiotensin and cocaine on the removal of noradrenaline* (10 *ng/ml*) *from the perfusion medium by isolated rabbit hearts*

Drug concentration ng/ml	Noradrenaline removal % ± s.e.	Rate of noradrenaline removal: ng/heart min^{-1} ± s.e.	Rate of noradrenaline removal: ng/g heart min^{-1} ± s.e.	n
Perfusion rate 25 ml/min, Tyrode, 34°				
Controls	41·1 ± 1·3	103·0 ± 3·2	23·4 ± 1·2	12
Angiotensin				
0·013	44·8 ± 2·2	112·1 ± 5·4	24·1 ± 1·4	5
0·13	41·5 ± 0·9	104·0 ± 2·3	23·3 ± 1·7	6
1·3	38·0 ± 2·2	95·1 ± 5·4	23·4 ± 1·0	5
13·0	39·5 ± 2·3	99·0 ± 5·7	20·7 ± 1·8	6
130·0	39·9 ± 2·2	100·0 ± 5·5	22·3 ± 2·1	6
13 000·0	33·2 ± 3·0†	83·1 ± 7·6†	18·5 ± 1·4†	5
Cocaine				
10 000·0	12·3 ± 3·6‡	30·8 ± 9·0‡	5·5 ± 1·3‡	3
Perfusion rate 10 ml/min, Tyrode, 34°				
Controls	66·1 ± 1·1	66·1 ± 1·1	17·1 ± 1·9	3
Angiotensin				
2·0	66·1 ± 2·3	66·1 ± 2·3	15·2 ± 1·3	4
Perfusion rate 10 ml/min, Krebs, 37°				
Controls	69·2 ± 1·7	69·2 ± 1·7	14·6 ± 1·1	6
Angiotensin				
2·0	69·4 ± 3·6	69·4 ± 3·6	13·4 ± 1·0	3
Cocaine				
10 000·0	9·0 ± 4·0‡	9·0 ± 4·0‡	2·1 ± 1·1‡	3

Experiments with Tyrode solution: 45 min perfusion with PSS; 8 min perfusion with angiotensin or cocaine; thereafter with angiotensin or cocaine + noradrenaline. Experiments with Krebs solution: 120 min perfusion with PSS; thereafter simultaneous perfusion with angiotensin or cocaine + noradrenaline.

* The amount eliminated expressed as per cent of the amount infused.

† Significantly different from corresponding controls ($P<0·05$).

‡ Significantly different from corresponding controls ($P<0·001$).

perfusion rate of 10 ml/min (Tyrode solution, 34°). The two values are significantly different ($P<0·001$). The total amount removed per g heart per min was, however, greater at the higher coronary flow ($P<0·001$). There was no significant difference caused by substition of Tyrode solution at 34° by Krebs solutions at 37°, at equivalent flow rates. Cocaine (10 μg/ml) reduced the loss of noradrenaline from the perfusate by 70% (perfusion rate 25 ml/min) or 87% (perfusion rate 10 ml/min). Angiotensin caused a much smaller but significant inhibition (19%) only at a concentration of 13 μg/ml.

Angiotensin and cocaine were also compared for their influence on the positive inotropic effect of noradrenaline. In hearts perfused at a rate of 25 ml/min, the peak systolic tension developed was 7·1 ± 0·3 g (n = 43). The increase in the strength of contraction caused by 10 ng/ml noradrenaline was 2·4 ± 0·5 g (n = 10). In the presence of cocaine, the effect of noradrenaline was increased (11·0 ± 0·1 g; $P<0·001$, n = 3). Angiotensin did not influence the inotropic action of noradrenaline (2·3 ± 0·3 g; n = 5) even in the very high concentrations necessary to reduce the removal of noradrenaline from the perfusion fluid.

Noradrenaline uptake

The mean noradrenaline content of 12 hearts perfused with Tyrode solution for 45 min was 1123 ± 99 (range, 606 to 1918) ng/g. The mean endogenous content was subtracted from the total amount found in hearts perfused with noradrenaline at high concentrations. In the experiments with 5 μg/ml, moreover, extracellular noradrenaline was not washed out at the end of the infusion. Extracellular space of rat hearts is 325 μl/g (Iversen, 1965); we used this value for correction.

The results are presented in Table 2. Neither metanephrine (5 μg/ml) nor angiotensin (130 ng/ml) significantly influenced the uptake at 5 μg noradrenaline/ml. After 10 min perfusion with 20 μg/ml noradrenaline and 2 min wash-out, the amount taken up and retained did not significantly differ in control hearts and those infused with metanephrine (20 μg/ml) or angiotensin (130 ng/ml).

Table 2. *Effect of metanephrine and angiotensin on the uptake of noradrenaline by the rabbit isolated heart*

Noradrenaline concentration	Drug	Noradrenaline uptake (ng/g ± s.e.)	n
	—	1708 ± 222	6
5 μg/ml*	Metanephrine 5 μg/ml	1318 ± 307	3
	Angiotensin 130 ng/ml	2166 ± 680	3
	—	3738 ± 133	8
20 μg/ml†	Metanephrine 20 μg/ml	3327 ± 588	3
	Angiotensin 130 ng/ml	3409 ± 249	6

Infusion of the drugs tested for inhibition of uptake started 8 min before noradrenaline infusion.

* Noradrenaline infused for 5 min. Values corrected for endogenous noradrenaline content and that of extracellular fluid.

† Noradrenaline infused for 10 min, followed by a 2 min wash-out. Values corrected for endogenous noradrenaline content.

DISCUSSION

The output of noradrenaline from isolated rabbit hearts during sympathetic nerve stimulation is enhanced by angiotensin (Starke & others, 1969). If the coronary flow is kept constant, significant augmentation is obtained by 130 pg/ml, and maximal effects by 1·3 ng/ml. In these concentrations, angiotensin is without any effect of the removal of infused noradrenaline from the perfusion medium. The ratio of the dose producing a small inhibition of removal of noradrenaline from the perfusion fluid (13 μg/ml) and that augmenting the output of noradrenaline on sympathetic stimulation is 10^5. On the other hand, cocaine, a typical blocker of noradrenaline uptake across the neuronal membrane, greatly reduced the removal of noradrenaline from the perfusion fluid in concentrations which enhance the output of noradrenaline during sympathetic nerve stimulation (10 μg/ml; Huković & Muscholl, 1962). It seems unlikely, therefore, that the cocaine-like action observed with excessive concentrations of angiotensin is related to the increased output of noradrenaline during sympathetic stimulation which is caused by low concentrations of angiotensin.

Recently, Peach & others (1969) demonstrated that the uptake of (±)-[^{3}H]noradrenaline into isolated perfused rabbit hearts is reduced by angiotensin; 0·05 ng/ml

caused a diminution by 50%, 2·0 ng/ml by 80%. As the elimination of infused noradrenaline from the perfusion medium is mainly the result of net-uptake into the heart (Lindmar & Muscholl, 1964), inhibition of net-uptake should be accompanied by the appearance of a greater amount of noradrenaline in the venous effluent. In hearts perfused with Tyrode solution at a rate of 25 ml/min, we did not find an effect on the removal of perfused noradrenaline by angiotensin even with doses of angiotensin much higher than those of Peach & others (1969). Moreover, the experimental conditions used were similar to those of these authors (Krebs solution, 37°, 10 ml/min, 2 h perfusion with PSS before the administration of drugs, simultaneous addition of 2 ng/ml angiotensin and 10 ng/ml noradrenaline). Cocaine, on the other hand, always caused a clearcut reduction of noradrenaline elimination.

The accumulation of labelled noradrenaline may be diminished, without a simultaneous increase of the amount of fluorimetrically determined noradrenaline in the perfusate, by several mechanisms. In the experiments of Peach & others (1969), (±)-[^{3}H]noradrenaline was diluted with unlabelled (—)-noradrenaline. Angiotensin might specifically inhibit the uptake of the (+)-isomer. This would result in a great decrease of the accumulation of labelled amine in the heart, but only a comparatively small increase of fluorimetrically determined noradrenaline in the venous effluent. It is not very likely, however, that the above-mentioned reduction of [^{3}H]noradrenaline uptake by 80% can be explained in this way (cf. Draskóczy & Trendelenburg, 1968).

Secondly, angiotensin might accelerate the metabolism of noradrenaline. The percentage of total myocardial radioactivity present as metabolites was not changed by the peptide (Peach & others, 1969). Apparently, metabolic products were not determined in the perfusate. That portion of noradrenaline which, in the presence of angiotensin, is neither retained in the heart nor recovered from the perfusate, may be contained in the effluent as metabolites.

Finally, the accumulation of [^{3}H]noradrenaline in the heart is the result not only of net-uptake, but also of an exchange with the endogenous stores. Exchange with endogenous noradrenaline is slow in rat hearts (Iversen, 1963), but it may be more rapid in rabbit hearts. Angiotensin might inhibit the exchange process without appreciably diminishing net-uptake. This would reduce the accumulation of [^{3}H]noradrenaline in the heart, leaving the amount of chemically determined noradrenaline in the venous effluent unaffected. Single and combined, these mechanisms may help to explain the results of Peach & others (1969) as well as our own.

In earlier reports, large doses of angiotensin were needed to demonstrate an inhibition of the uptake of [^{3}H]noradrenaline (Panisset & Bourdois, 1968; Palaić & Khairallah, 1967a,b). Angiotensin (1 ng/ml) did not inhibit the elimination of (—)-noradrenaline from the perfusion medium by the cat spleen (Thoenen, Huerlimann & Haefely, 1965); intravenous infusion into pithed rats at a rate of 30 ng/kg min^{-1} did not influence the uptake of (±)-[^{3}H]noradrenaline by several organs (Pals & Masucci, 1968).

The drug sensitivity of the uptake of noradrenaline at very high concentrations ($Uptake_2$) differs from that of $Uptake_1$ (Iversen, 1965). Therefore, we examined the influence of angiotensin and metanephrine on $Uptake_2$. Metanephrine is a potent blocker of $Uptake_2$ in the rat heart (Iversen, 1965). In our experiments, the accumulation of noradrenaline was only insignificantly reduced. It is known that metanephrine does not uniformly decrease the uptake of noradrenaline; it increases for

instance the uptake into the submaxillary gland of rats *in vivo* (Iversen, Fischer & Axelrod, 1966). The uptake of noradrenaline was unaffected by 130 ng/ml of angiotensin, i.e. 10^3 of the dose increasing the output during sympathetic nerve stimulation. Inhibition of an $Uptake_2$-like mechanism, therefore, does not seem to be responsible for the potentiating effect of angiotensin on noradrenaline output.

Zimmerman & Gisslen (1968), comparing the effects of angiotensin and cocaine, concluded that the peptide did not increase noradrenaline outflow by a cocaine-like mechanism. They assumed that the amount of noradrenaline liberated per nerve impulse might be augmented. Our results support their conclusion. At high concentrations of noradrenaline, net-uptake is unaffected by angiotensin. At low concentrations of noradrenaline, a cocaine-like effect can be obtained by very high doses of angiotensin, but it is absent at the low doses which increase the output of transmitter during sympathetic nerve stimulation.

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