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SPECIFIC SUPERSENSITIVITY OF SMOOTH MUSCLE TO ANGIOTENSIN II AFTER NEPHRECTOMY

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1 The dose-response curves for angiotensin, 5-hydroxytryptamine and carbachol were obtained on rat uterus and colon excised 24 h after a bilateral nephrectomy or a sham operation.

2 There was a shift to the left in the dose-response curves to angiotensin of organs from nephrectomized animals. Dose-response curves to 5-hydroxytryptamine and carbachol were not affected by nephrectomy.

3 The interaction of the two competitive antagonists (1-Asn, 5-Ileu, 8-Ala)-angiotensin II and (1-Asn, 5-Ileu, 8-Ileu)-angiotensin II with angiotensin II receptors was tested in control and nephrectomized organs. There was a decrease of the K_I values in colon muscle after nephrectomy.

4 In conclusion, nephrectomy produces a specific supersensitivity to angiotensin that seems to be due to a change in the structure of angiotensin receptors, at least in colon smooth muscle. This increase in sensitivity may be the result of the elimination of a tropic factor that acts on angiotensin receptor sites.

Introduction

It has been repeatedly shown that bilateral nephrectomy increases the pressor response of experimental animals to either renin or angiotensin (Page & Helmer, 1940; Houssay & Dexter, 1942). The possible mechanism of this hypersensitivity was not explored until recently. Stouder & Wathen (1972) studied the change of the pressor response to angiotensin and of the ionic composition of the plasma in rats after nephrectomy and ureteral ligation. These authors found an increased response after either procedure. Since there was a coincident rise of plasma K^+ concentration with both procedures they suggested that this could be the cause of the increased effect of the hormone.

It was of interest to test whether bilateral nephrectomy was associated with a change in the response of isolated smooth muscles to angiotensin and other spasmogens. With this purpose, experiments were performed with rat colon and uterus, isolated from either normal animals or from animals nephrectomized bilaterally 24 h before the experiments.

Methods

The animals used in these experiments were male or female Wistar rats of about 150 g weight. They were anaesthetized with ethylmethylbutyl-barbiturate (Mebubarbital, Abbott, France) 30 mg/kg. In some of them a bilateral nephrectomy was performed. The remaining animals were sham operated, and they constituted the control group. Twenty-four hours after the operation they were killed by a blow on the head, and the uterus or colon excised. The methods used for the preparation of the organs, the exposure to agonist and antagonist drugs and statistical analysis were described previously (Papadimitriou & Worcel, 1974).

The concentrations of angiotensin (AtII) giving 50% maximal responses (ED_{50}) in control animals were $1.5 \pm 0.3 \times 10^{-9}$ M (11 experiments) in rat colon and $1.2 \pm 0.1 \times 10^{-8}$ M in rat uterus (12 experiments), values that do not differ significantly from those previously reported (Papadimitriou & Worcel, 1974).

Drugs

(5-Valyl)-angiotensinamide (Hypertensin, Ciba Pharmaceutical Company, Summit, N.J., U.S.A.), 5-hydroxytryptamine sulphate H_2O (Mann Research Laboratories Inc., New York, U.S.A.), carbamylcholine (Carbachol, Sigma Chemical Co., St-Louis, Mo.), ethylmethylbutyl barbiturate (Mebubarbital, Abbott, France). The angiotensin II analogues were provided by Drs F.M. Bumpus and

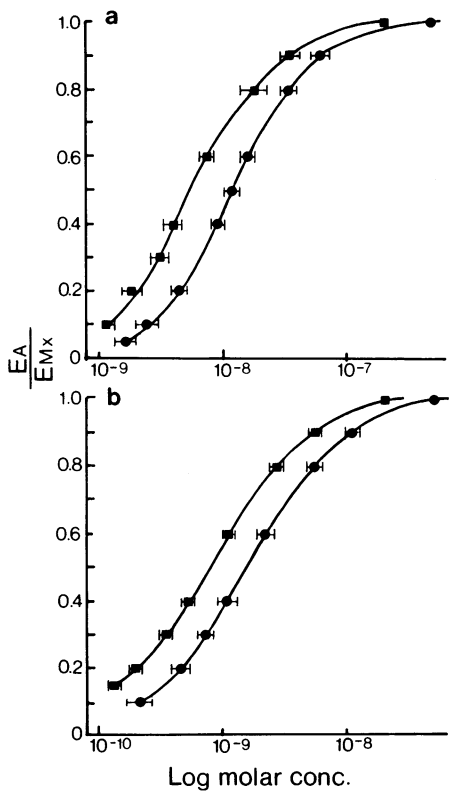

Fig. 1 Log dose-response curves to angiotensin II on (a) uterus and (b) colon from control animals (•) and on organs from nephroctomized animals (▪). This response is expressed as a fraction of the maximum effect produced by angiotensin in each experiment (E_A/E_{Mx}). The average dose necessary to obtain a certain response was calculated. Horizontal bars show the standard errors. The curves are fitted by hand and represent the result of 10 to 12 experiments.

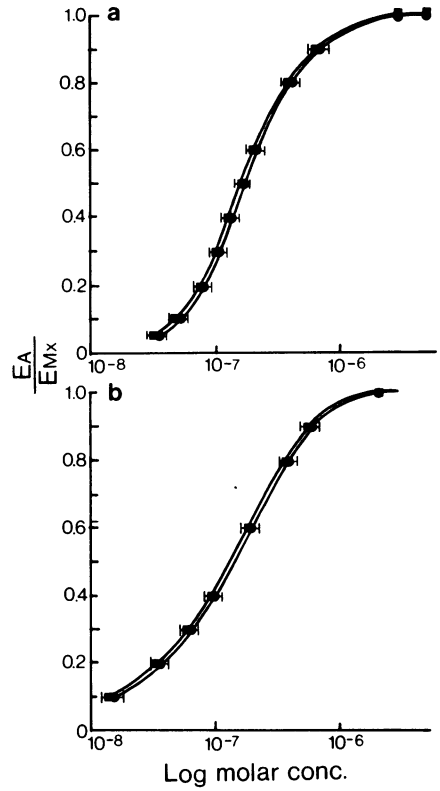

Fig. 2 Log dose-response curves to (a) 5-hydroxytryptamine and (b) carbachol in rat uterus and colon, respectively. Data plotted as in Figure 1. The curves represent the results of 10 to 11 experiments.

P.A. Khairallah: (1-Asn, 5-Ileu, 8-Ileu)-angiotensin II, and by Drs F.M. Bumpus, P.A. Khairallah and D. Regoli: (1-Asn, 5-Ileu, 8-Ala)-angiotensin II.

Results

Effect of nephrectomy on dose-response curves to angiotensin in rat colon and uterus

After bilateral nephrectomy there was a marked increase in sensitivity of both preparations to angiotensin II, the ED_{50} values being $7.5 \pm 0.1 \times 10^{-10}$ M (rat colon, 12 experiments) and $5.4 \pm 0.1 \times 10^{-9}$ M (rat uterus, 10 experiments), see Figure 1. This displacement of dose-response curves to the left is highly significant ($P < 0.001$).

This effect of nephrectomy could result either from a specific change in the receptors or it could be a non-specific effect of the multiple humoral changes that follow this operation. It has been suggested that hyperkalemia and hyponatremia and the associated ionic shifts could produce the increased pressor response to angiotensin after nephrectomy and ureteral ligation (Stouder & Wathen, 1972). The effect of the change in ionic composition of the extracellular fluid is unlikely to account for our results, since the strips were tested after at least 1 h equilibration with physiological salt solution.

In order to exclude any non-specific supersensitivity of smooth muscle the response of uterine muscle to 5-hydroxytryptamine and of the colon to carbachol was tested in organs excised from control and nephrectomized animals. In neither case was the sensitivity altered after nephrectomy (Figure 2).

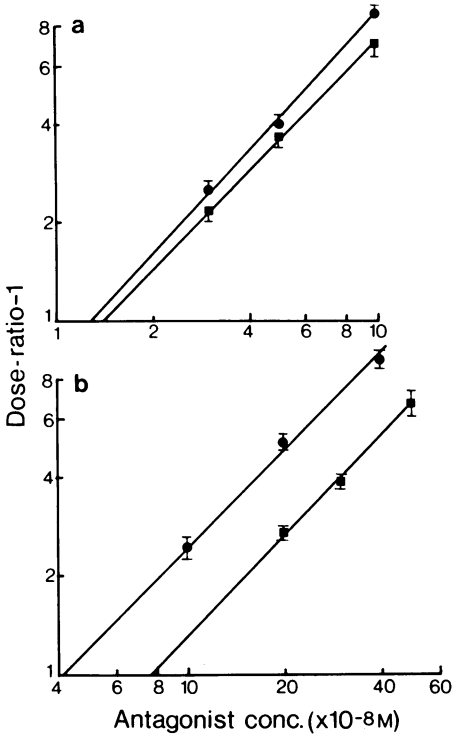

Fig. 3 Antagonism of angiotensin II by (1-Asn, 5-Ileu, 8-Ala)-angiotensin II. Logarithmic plots of (dose-ratio − 1) against concentration on (a) rat uterus and (b) colon. Organs from control animals (•); organs from nephrectomized animals (▪). Each point is the mean of 7 to 10 experiments. The concentration at which the line cuts the dose-ratio − 2 line gives the dissociation constant for the antagonist (K_I).

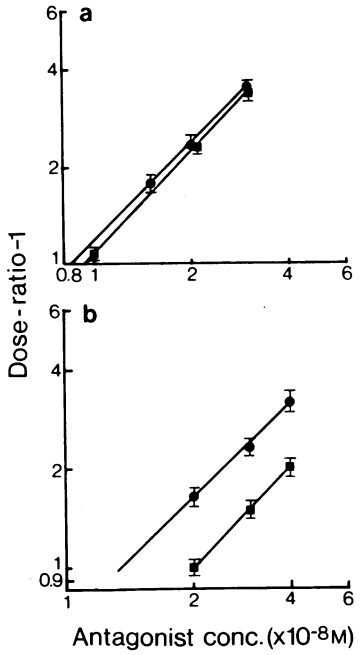

Fig. 4 Antagonism of angiotensin II by (1-Asn, 5-Ileu, 8-Ileu)-angiotensin II. Data plotted as in Figure 3. Each point is the mean of 8 to 12 experiments.

Effect of nephrectomy on the action of angiotensin agonists

The displacement of angiotensin dose-response curves does not mean necessarily that there is an increase in the affinity of angiotensin for its receptors, since ED_{50} is not necessarily equal to the real dissociation constant (Stephenson, 1956). The relation between receptor occupancy and response may be non-linear (Nickerson, 1956; Ariëns, Simonis & Van Rossum, 1964), and the shift to the left of the concentration-effect curves could be the result of an increase in receptor reserve due to an increase in the number of receptors after the reduction or disappearance of circulating AtII after nephrectomy. To test whether the binding characteristics of the receptors had been altered two competitive antagonists were used. It has already been shown that (1-Asn, 5-Ileu, 8-Ileu)-angiotensin II (Bumpus, Khairallah & Smeby, personal communcation) and (1-Asn, 5-Ileu, 8-Ala)-angiotensin II (Türker, Yamamoto, Khairallah & Bumpus, 1971) are competitive antagonists. As can be seen in Fig. 3, after nephrectomy there is a significative decrease of the affinity of colon smooth muscle to (1-Asn, 5-Ileu, 8-Ala)-angiotensin II. The K_I values obtained were $3.6 \pm 0.1 \times 10^{-8}$ M in controls (9 experiments), and $7.4 \pm 0.1 \times 10^{-8}$ M after nephrectomy (7 experiments); $P < 0.001$. On the other hand there was no change in the affinity of the same antagonist in uterine smooth muscle. The K_I values were $1.2 \pm 0.1 \times 10^{-8}$ M in controls (10 experiments) and $1.4 \pm 0.1 \times 10^{-8}$ M after nephrectomy (13 experiments).

Figure 4 shows that quite similar results were observed with (1-Asn, 5-Ileu)-angiotensin II. There was no change in the action of the antagonist on uterine smooth muscle after nephrectomy. The K_I values were $0.8 \pm 0.1 \times 10^{-9}$ M in controls (8 experiments) and $0.9 \pm 0.1 \times 10^{-3}$ M (10 experiments) after nephrectomy. On the other hand, as in the previous case, there was a significant decrease of affinity in colon smooth muscle after nephrectomy. The K_I values were $1.2 \pm 0.1 \times 10^{-8}$ M in controls

(12 experiments) and $2.0 \pm 0.1 \times 10^{-8}$ M after nephrectomy (9 experiments); $P < 0.001$.

Discussion

Nephrectomy produces supersensitivity to AtII, characterized by a shift to the left of the dose-response curves in both uterine and colon smooth muscle. The supersensitivity to angiotensin may be secondary to a change in the steric structure of angiotensin receptors. The observed variations in ED_{50} values do not by themselves mean that nephrectomy changes the affinity of angiotensin receptors for angiotensin II (Papadimitriou & Worcel, 1974), and the study of the action of competitive antagonists, which does not involve other steps beyond the occupation of receptor sites (Waud, 1968), was carried out in order to examine this point. The results obtained with 8-Ileu- and 8-Ala-angiotensin II show that in colon smooth muscle K_I values are higher after nephrectomy, and strongly suggest a change of angiotensin receptors, since in the case of competitive antagonists, the dissociation constant (K_I) should be similar in different organs and with different agonists if receptors were identical (Schild, 1957). A steric change of the receptors in uterine muscle cannot be excluded on the basis of the absence of variation of K_I, since a change of conformation might also occur without changing their affinity to some antagonists.

The explanation of the supersensitivity induced by nephrectomy is difficult, and these experiments give no information about the mechanism involved. Nephrectomy may affect the response of smooth muscle receptors to angiotensin either as a consequence of a diminution of renin and angiotensin concentration in blood (Worcel, Meyer, Anglès d'Auriac & Milliez, 1969) or by some other mechanism not related directly to angiotensin blood levels.

The phenomenon described resembles the denervation supersensitivity of skeletal muscle (Miledi, 1960a, b). Jenkinson (1960) found that the affinity of the receptors for (+)-tubocurarine was increased after denervation, a result comparable to the present findings that the affinity for angiotensin antagonists for the receptors in rat colon is enhanced.

Sayers & Beall (1973) have recently described what they call a detropication hypersensitivity. They observed an increased production of 3′,5′-adenosine monophosphate (3′,5′-AMP) by isolated adrenal cortex cells from hypophysectomized rats, when stimulated with adrenocorticotrophic hormone (ACTH). As in our case, this increased 3′,5′-AMP synthesis is characterized by a marked shift to the left of the dose-effect curves, with a corresponding decrease in the ED_{50} to ACTH after hypophysectomy.

Supersensitivity to angiotensin II after bilateral nephrectomy seems to be another example of the more general phenomenon of increased sensitivity of the target cell to the mediator or hormone after elimination of its natural source. It is probably due to a change in the structure of angiotensin receptors, at least in colon smooth muscle.

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