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Noradrenaline as a Possible Mediator of Angiotensin II Induced Fluid Transport in Rat Ileum *in vitro*

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In isolated segments of ileum excised from bilaterally adrenalectomized and nephrectomized rats, 10^{-12} M angiotensin or 10^{-3} M noradrenaline added to serosal medium stimulated both fluid transfer and NaCl transport. The alpha adrenergic antagonist phentolamine blocked the stimulation of fluid transfer induced by angiotensin. These results are consistent with the hypothesis that noradrenaline may mediate the increase of intestinal fluid absorption induced by angiotensin in the rat. In segments of isolated ileum from normal rats 10^{-12} M angiotensin only stimulated fluid transfer under one of the two following conditions when 10^{-3} M imipramine, a noradrenaline uptake blocker, was present in the serosal medium; or when the rats had been previously treated with L-Dopa, a precursor of noradrenaline biosynthesis. These results suggested that the necessity for bilateral adrenalectomy and nephrectomy might be associated to the necessity of increasing the tissue levels of noradrenaline. Direct measurement of noradrenaline tissue content confirmed this.

Fluid transport by the intestine is stimulated by 10^{-12} M angiotensin II both *in vivo* (1, 8) and *in vitro* (2, 3, 6). Results obtained from *in vitro* experiments, usually with stripped epithelia, have generally been interpreted to show that angiotensin directly stimulates the epithelial cells and their transport activity (2, 3, 6). However, LEVENS *et al.* (8) suggest that the stimulus of transport by angiotensin *in vivo* is possibly mediated by the noradrenaline release from the sympathetic nerve-endings. Another significant difference between the *in vivo* and *in vitro* situations is that *in vitro*, in order to obtain the response to angiotensin the intestinal epithelium must be from an animal previously sensitized to angiotensin by means of bilateral adrenalec-

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tomy and nephrectomy (2, 3, 6), whereas for *in vivo* work, normal intact animals may be used (1, 8).

Materials and Methods

Male Wistar rats weighing between 200 and 300 g were used in all experiments. Bilateral adrenalectomized and nephrectomized (AXNX) rats were used in this study. Bilateral adrenalectomy and nephrectomy was carried out under ether anaesthesia 24 h before the experiments.

Measurement of rat ileum fluid transport and the net fluxes of sodium and chloride followed the method described in detail by DÍEZ DE LOS RÍOS *et al.* (3). The rinsing solution and the bathing medium had the following composition in mM: NaCl, 118.5; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25, pH was adjusted to 7.4. Solutions were bubbled with 95 % O_2 and 5 % CO_2. The bathing medium was maintained at 38 °C with a thermostatic bath. The fluid transfer rate (J_v) is given as μl/h per 100 mg wet tissue and was determined by weighing the cannulated sacs before and after the incubation. The sodium and chloride net fluxes were measured by analysing the fluid content of the sacs. Sodium and chloride net fluxes are given as μEq/h per 100 mg wet weight.

Measurement of ileum noradrenaline content was performed following the method described in detail elsewhere (11). Fluorescence measurements were made with a Perkin-Elmer 650-105 spectrofluorimeter at an excitation wavelength of 368 nm and 505 nm emission wavelength.

Angiotensin (Hypertensine; Ciba) was diluted with the incubation medium to give a concentration of 10^{-12} M and was introduced into the serosal medium. Noradrenaline (Sigma) was diluted with the incubation medium to give a final concentration of 10^{-3} M before being introduced into the serosal medium. Imipramine (Ciba), was used at 10^{-3} M in the serosal medium and L-Dopa (Fher) at 2.5 mg i.p. every 8 hours, for three days prior to experiments.

Results

The effect of angiotensin and noradrenaline on fluid transport by the ileum of bilateral adrenalectomized and nephrectomized (AXNX) rats is shown in table 1. Angiotensin increased fluid transfer as well as the net sodium and chloride fluxes. The obtained results are consistent with an increase in NaCl transport stimulated by the presence of angiotensin. Qualitatively similar results were obtained in the presence of noradrenaline. This similarity suggests that the *in vitro* action of angiotensin is possibly mediated by noradrenaline release from sympathetic nerve-endings. To explore further this possibility, the effect of the alpha-blocker phentolamine on the intestinal response to angiotensin was studied. Fluid transfer by the ileum of AXNX rats increased ($P < 0.01$) in five experiments from 28 ± 7 μl/h per 100 mg to 76 ± 18 μl/h per 100 mg in the presence of angiotensin. The simultaneous presence of angiotensin and phentolamine in the bathing medium reduced ($P<0.01$) the fluid transfer to 11 ± 5 μl/h per 100 mg. These results show that the stimulatory action of angiotensin on fluid transfer is blocked by phentolamine.

If the angiotensin action is mediated by noradrenaline, this would explain the necessity for employing intestinal segments from bilateral adrenalectomized and nephrectomized rats to elicit the transport increase response to angiotensin *in vitro*. Bilateral adreno-nephrectomy would produce an accumulation of noradrenaline in the tissues and therefore, angiotensin would induce a larger re-

Table I. *Effects of angiotensin and noradrenaline on fluid transfer (J_v), sodium transport (J_{Na}) and chloride transport (J_{Cl}) in segments of AXNX rat ileum.*
Mean values ± S.E.M. are given. The number of tissues studied is given in parenthesis. Paired t-test was used.

	J_v (μl/h/100 mg)	J_{Na} (μEq/h/100 mg)	J_{Cl} (μEq/h/100 mg)
Control	20 ± 4 (11)	2 ± 1 (6)	3 ± 1 (6)
Control + angiotensin	53 ± 9 (11)**	5 ± 1 (6)**	6 ± 1 (6)**
Control + noradrenaline	63 ± 7 (11)**	12 ± 2 (6)**	11 ± 2 (6)**

** $p < 0.001$.

lease of noradrenaline in the ileum of AXNX rats. If this working hypothesis is correct, the angiotensin stimulation of rats ileum transport would occur in animals treated to increase the noradrenaline levels at the synapse gap by blocking its metabolism or facilitating its synthesis. To test this hypothesis normal animals were treated either with imipramine, a blocker of noradrenaline uptake, or with L-Dopa a precursor of noradrenaline in its biosynthesis. Results in table II show that in both cases stimulation of transport by angiotensin was observed. Finally, direct measurements of noradrenaline in ileal tissues were made. The noradrenaline content in normal rat ileum was 3.3 ± 1.0 μg per 100 mg; in AXNX rats this increased to 6.9 ± 1.1 μg per 100 mg ($P < 0.05$) which lends direct support to the working hypothesis.

Discussion

The stimulatory action of 10^{-12} M angiotensin on fluid transfer by segments of ileum from AXNX rats reported in the present study agrees well with the results of previous studies on rat intestine (1-3, 6, 7). It has been suggested that angiotensin increases fluid transfer by means of stimulating a neutral or coupled NaCl transport (3, 9, 10). Results presented in this paper are consistent with this hypothesis.

FIELD and MCCOLL (4) have shown that noradrenaline may stimulate NaCl transport by rabbit ileal mucosa. There are both alpha-adrenergic and beta-adrenergic receptors in the intestine. Only the α-adrenergic receptors are involved in the stimulatory response induced by noradrenaline in the ileum (4). The

Table II. *Effect of angiotensin on fluid transfer (J_v) in normal rat ileum segments.*
Imipramine was present in the serosal solution at 10^{-3} M concentration. L-Dopa was administered *i.p.* to the animals for three days before experiments. Mean values ± S.E.M. are given. The number of segments studied is given in parenthesis. Paired t-test was used.

Group	J_v (μl/h/100 mg) Normal	+ Imipramine	+ L-Dopa
Control	76 ± 13 (6)	34 ± 3 (6)	18 ± 7 (7)
Control + angiotensin	59 ± 9 (6)	78 ± 11 (6)*	73 ± 13 (7)**

* $p < 0.005$, ** $p < 0.001$.

results presented in this paper agree well with the reported stimulation of NaCl transport by noradrenaline (4). The present study shows that the increases in fluid transfer rates and the net sodium and chloride fluxes produced by angiotensin and noradrenaline are very similar. In addition, the angiotensin induced increase of transport was inhibited by the alpha blocker phentolamine. This suggests that the *in vitro* action of angiotensin upon intestinal function is possibly mediated by the release of noradrenaline from sympathetic nerve-endings. This hypothesis was first suggested by LEVENS *et al.* (7) working *in vivo*.

In rats pre-treated with reserpine to deplete the noradrenaline content of the post-ganglionic neurons, the angiotensin induced increase in fluid transfer, *in vivo*, has not been found (8). This observation suggests that the accumulation of high levels of noradrenaline at the nerve-endings is necessary to ensure the transport increase produced by angiotensin *in vitro*. The present experiments show that treatment with imipramine or L-Dopa to elevate the noradrenaline levels in the synapse gaps potentiates the action of angiotensin on intestinal transport. This might explain why previous bilateral adrenalectomy and nephrectomy are needed: the prior ablation of these organs increases the levels of noradrenaline in the intestinal neurons to potentiate the angiotensin action. Its actual occurrence, was confirmed in this study by direct measurement of noradrenaline levels in the ileum segments.

The hypothesis of a direct action on the epithelia by angiotensin *in vitro* is mainly based on experiments made on stripped intestinal preparations (2, 6). Unfortunately, these authors do not present histological evidence to show that Meissner's and Auerbach's plexuses were completely removed from their preparations. It is quite possible that after stripping, the submucosal, or Meissner's plexus remains in the preparation. In regard to this point GABELLA (5) has shown that about 5/6 of the total noradrenaline content is present in the submucosal plexus. Thus, even in a stripped preparation, high levels of noradrenaline may occur in tissues close to epithelial cells. It may be argued that it seems unlikely that the few ganglia remaining in the tissue may release enough noradrenaline to increase fluid transfer (3). However, as shown in the results section, the AXNX operation increases the noradrenaline content of the whole intestinal segment. This possibly potentiates fluid transfer stimulation by angiotensin in the ileum of AXNX rats.

Resumen

La angiotensina 10^{-12} M en el medio serosal de íleon aislado de ratas adrenalectomizadas y nefrectomizadas bilateralmente estimula tanto el transporte de fluido como el de NaCl. Efectos cualitativamente similares se observan con noradrenalina 10^{-5} M. La fentolamina bloquea la estimulación del transporte de fluido por la angiotensina. En segmentos aislados de íleon de ratas normales la angiotensina 10^{-12} M solamente estimula el transporte de fluido si la imipramina 10^{-5} M está presente en el medio serosal, o si las ratas han sido tratadas previamente con L-Dopa. Estos resultados sugieren que la necesidad de adrenalectomía y nefrectomía bilateral pueda estar asociada a la necesidad de incrementar los niveles tisulares de noradrenalina. La medida directa de los niveles tisulares de noradrenalina confirmó este punto.

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