

Motility of the Rat Ureter *in vitro*. Responses to Cholinergic Drugs

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The motility of the rat ureter has been studied *in vitro*. Sixteen per cent of isolated rat ureters present spontaneous motility which depends on the electrolytic composition of the nutritious solution employed. It seems that the cholinergic component in organs from animals previously reserpinized acts as stimulator since 56.6 % of ureters with spontaneous motility was observed *in vitro*.

The action of nicotine proves the existence of stimulating nicotinic receptors in the ureter wall. Acetylcholine acts as excitator as increases peristaltic frequency. Atropine, likewise, proves the existence of stimulating muscarinic receptors. Urine dilutions of 1:100 have also proves their ability for producing peristaltic motility *in vitro*.

Evidently the ureter presents a rich intramural vegetative innervation, as confirmed by studies carried out on man (8) in cats (7), in guinea-pigs (6) and in rats (2, 3, 15). Nevertheless, it still is not clear what role this intrinsic innervation may play. Nearly all the studies carried out to date emphasize the study of the adrenergic innervation, being very few those who have studied the cholinergic innervation. Our study has been focused on covering this field, pinpointing the possible existence of muscarinic receptors as well as nicotinic ones in rat ureter.

Materials and Methods

Experiments has been carried out on preparation obtained from male Sprague-Dawley rats, weighing 200 ± 15 g.

The animals were killed by a hit on the head and deblooded. Later by means of a wide an extensive incision in the abdominal wall, and after separating the viscera, the ureters were observed retroperitoneally, proceeding to their dissection in two stages. First, the kidney together with the ureter, placing both of them in a Petri dish containing Broom-Clark so-

lution at room temperature. Secondly, the ureter was separated from renal parenchyma, leaving only a portion which included the pelvis and renal calyx. At that moment the ureter was carefully separated from all its surrounding fat. The preparation was placed in a Magnus bath (10 ml) and was subject to an initial tension of 500-600 mg, leaving it to stabilize for a minimum of 30 minutes. The register of the motility was carried out by an isometric tension transducer Hewlett-Packard type FTA-100/1.

The animals were divided into two groups: Control group (162 animals) without any treatment, and a treated group (16 animals) which received reserpine (Ciba). The animals treated with reserpine as carried out according to the AMBACHE and ZAR procedure (1).

In both groups the response of the ureter preparations to the acetylcholine (Fluka) 1, 2.5, 5 and 10 $\mu\text{g/ml}$ and its antagonism to atropine (Merck) (0.1 and 1.0 $\mu\text{g/ml}$) were studied, as well as the responses to different doses of nicotine (Fluka) (dilutions of 1×10^{-1} and 1×10^{-4}).

In organs of the control group the response of the preparations to the animal's urine (extracted by bladder puncture after sacrificing) was studied. Three parameters have been considered: Frequency (number of contractions/minute), tension or amplitude (in mg of strength, measurable by this displacement of the wave in mm) and duration of the contraction in seconds.

All the data were submitted to statistical treatment, calculating for each point its average value and standard deviation. In those cases in which it was necessary the Student *t* was used in order to compare the samples.

Results

Spontaneous motility. The electrolytic composition of the nutritious solution in-

fluenced greatly the contractile response of the isolated rat ureter. The Broom-Clark's solution has been used because it gives the most homogenous results. Within the preparations tested, not all presented spontaneous motility, once placed in the bath. In the control group only 16.6 per cent of the organs tested presented spontaneous motility, which appears 20-50 minutes after immersion to the bath. On the other hand, the treated group showed spontaneous motility in 56.2 per cent of the cases, immediately after immersion.

Effects of the acetylcholine. In the control group of animals the characteristic response to acetylcholine, for doses of 2.5, 5 and 10 $\mu\text{g/ml}$ is a train of waves, which develop after applying only one dose and which is maintained during more than 30 minutes of recording. The only parameter which decreases with time is the frequency by gradually increasing the interval between each wave of contraction, until the response wanes completely. The minimum dose of acetylcholine capable of provoking some isolated response was 0.5 $\mu\text{g/ml}$.

The response developed by acetylcholine is antagonized completely by atropine, at doses between 0.1 and 1.0 $\mu\text{g/ml}$.

In the treated group with reserpine the response of the preparation has the same characteristics as the control group, except that the frequency as well as the amplitude were now clearly potentiated. Whilst in the control group the atropine totally antagonized the effects of the acetylcholine, in the group treated there was only a partial antagonism (fig. 1).

Effects of the nicotine. The addition of nicotine to the bath has been carried out at different doses: 1×10^{-1} and 1×10^{-4} dilutions from nicotine (—). In the control group, the addition of nicotine to the bath diluted to 1:10 provokes in a few seconds a rather irregular hyperperistalsis effect which rapidly, within the first

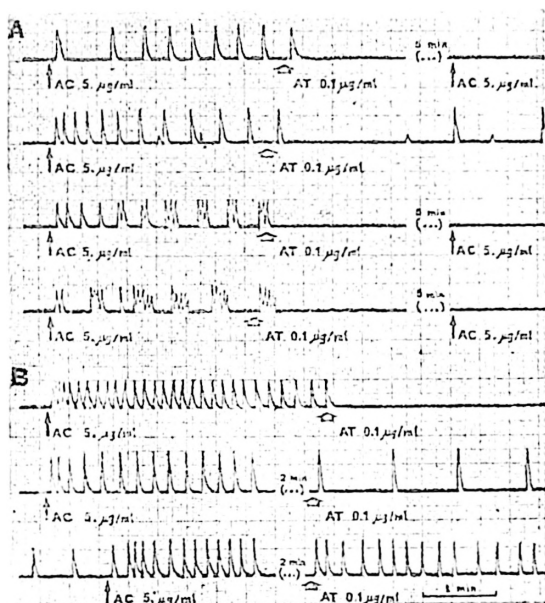


Fig. 1. Motility of rat ureter in vitro. A): Effect of acetylcholine (AC) in the control group and in the one previously treated with reserpine. B): Whilst in the control group the atropine (AT) totally antagonized the effects of acetylcholine, in the treated group they were only partial antagonism.

minutes of adding the dose, provokes a complete cessation of all activity. Sometimes, immediately after ceasing the activity, appears again by means of consecutive washings. The addition to bath of 1:10,000 dilutions provoke the development of peristaltic activity, with the following characteristics: The response is not always immediate. Sometimes it is delayed 1-2 minutes. In organs with spontaneous motility, nicotine increases the frequency (in first two minutes), the amplitude and also the duration of the contractions.

The latter, reaches progressively higher values. In organs without spontaneous motility the nicotine equally produces peristalsis, with the same characteristics as in the previous case. After consecutive washings, a motility component is nearly always conserved, with waves less am-

plitude, which resemble those of spontaneous motility. If a second dose is tested again, the same as the firsts, the organ is stimulate again, giving a motility of similar characteristics. In the group treated with reserpine the results are fairly similar nevertheless observing some differences. So, the force of contraction or amplitude of the wave regards to the spontaneous motility in the same group has little significance, as oppose to that which occurs in the control group in the control group. With regards to the duration of the wave, in the majority of cases from the second minute onward a progressive increase become apparent.

Effects of the urine. Experiments were carried out in a group of five animals of the control group, in which the urine has been added at dilutions of 1:100. Have been shown to be capable of develop peristaltic motility, at the same time as increasing the force of contractions and the wave duration. This motility developed by adding the animal's urine to the bath was not influenced at all by the ulterior addition of atropine (0.1 µg/ml).

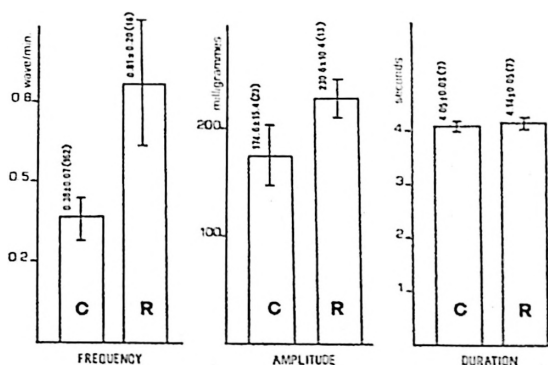


Fig. 2. Values of parameters in ureteral motility.

Control group (C) and the one previously treated with reserpine (R). In bracket, number of the experiments. In the frequency, $t = 1.93$ and $p < 0.05$; in the amplitude $t = 2.93$ and $p < 0.01$; in duration, $t = 1.26$ and N.S.

Discussion

The method utilized in order to register the ureter activity *in vitro* with the isometric techniques enables to study all the ureter tract in small animals. In this survey in the rat only in 16.6 per cent of all organs studied spontaneous motility was present when put into the bath, the rate was 2.02 ± 0.07 waves/min. However, if those organs which do not present motility were included, these rates were 0.38 ± 0.07 waves/min. This points out that these results are different from other autor (13) and also that they were carried out with other techniques *in situ* (14).

This difference can be explained by the fact that in one way the technique utilized was different and in another way the experimental conditions were not the same, they included the organs all together presenting or not motility.

In the treated group there are an increase of spontaneous motility (56.2 per cent) the rate is 0.81 ± 0.2 waves/min that is very significant with respect to the group of animals that were not treated ($p < 0.05$). Acetylcholine provokes an increase in the frequency of contraction, as well as, the amplitude of the wave, that is not demonstrated *in situ* (1). Many of these results are in accordance with those observed in the dog's ureter (4, 9, 11).

This results with nicotine are in accordance with those found by other authors, who pointed out hyperperistalsis (5). The effect of urine added to the bath corroborates the fact supported by others authors, in that urine could be a main factor that provokes the excitation and nervous transmission of the ureter (12). We think now that the problem is the following: The action is induced by urine's osmotic composition or by some active products in the urine?

Resumen

El uréter aislado de rata presenta en un 16,6 % de todos los casos estudiados motilidad

espontánea, la cual se halla influida por la composición electrolítica del medio nutricio empleado. Animales tratados previamente con reserpina presentan motilidad espontánea en un 56,6 % de lo que cabe deducir que el componente colinérgico es excitador. La acción de la nicotina prueba la existencia de receptores nicotínicos estimulantes en las paredes del uréter, así como la acción de la acetilcolina y su antagonismo por la atropina señalan la existencia de receptores muscarínicos.

Diluciones de orina del propio animal (1: 100) son capaces de desencadenar la actividad peristáltica en el uréter aislado de rata.

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