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BRIEF COMMUNICATION

Angiotensin-Induced Drinking: Sexual Differences[1]

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VIJANDE, M., M. COSTALES, O. SCHIAFFINI AND B. MARIN. *Angiotensin-induced drinking: Sexual differences.* PHARMAC. BIOCHEM. BEHAV. 8(6) 753–755, 1978. – The dipsogenic effect of Angiotensin-II (A-II) in relation to sexual variables was studied. It was found that Angiotensin-II administered SC constitutes a stimulus which induces more drinking in females than in males. Moreover, the adult females show maximum sensitivity to A-II during proestrus. The males and females castrated at birth, and females androgenized at birth, drink similar volumes of water after A-II SC injection. The pattern of stimulated intake is different in the two sexes, and appears to depend upon the development of the rats.

Angiotensin-II Subcutaneous injection Drinking Sex-differences

THE OCTAPEPTIDE Angiotensin-II is a substance with a strong dipsogenic action, a "thirst hormone", directly implicated in ingestive behavior [5]. Its action is fundamentally related to extracellular fluid depletion [8]. A-II acts as dipsogen in the rat when it is injected endovenously [9], subcutaneously [6], and intracerebrally [3, 7, 15]. It induces many species to drink including the cat [2] and the monkey [13]. The possibility that A-II can pass through the blood brain barrier (BBB) has been discussed, and the existence of components of the renin-angiotensin system in brain has been demonstrated [7]. The preoptic area seems to be one of the sites most sensitive to the action of A-II [4], and possible substratum of the hormone to induce thirst, when it is injected peripherally. It has been reported that extensive lesions of the preoptic lateral area inhibit the thirst induced by A-II when it is peripherally injected [14]. The anterior hypothalamus and preoptic areas are equally involved in the regulation of sexual function [12], and differentiation [11].

It was the purpose of the present investigation to study the relationship between sexual differentiation and the dipsogenic effect of the A-II.

METHOD

Animals and Surgery

One hundred eighty-seven Wistar rats (81 males and 106 females) weighing 200–220 g were used in the experiments. The animals were fed standard pellets, and watered ad lib. The laboratory was on a light cycle of 12h L–12 h D. Ten days before the experiments, the animals were kept in individual cages. The females were classified into the following groups: 16 androgenized at birth; 9 castrated at birth, 19 perpuberally and 20 in adulthood; 20 in diestrus, 15 in estrus and 11 in proestrus. The males were classified as follows: 25 castrated at birth, 11 prepuberally, and 23 in adulthood; finally 23 intact adults. The animals were castrated under sodium pentobarbital (Nembutal, 40 mg/Kg IP) anesthesia. Castration at birth was performed under hypothermia, produced by placing the animals into the freezer for a sufficient time. The sexual cycle of the females was tested by vaginal smear during four complete cycles. The androgenization of the females was carried out by injecting 5 mg Propionate of Testosterone subcutaneously, before the 3rd day of life.

Procedure

On the day of the experiment the food was withdrawn at midday, and before injecting the animals, a bottle filled with unfiltered tap water replaced the normal drinking apparatus. The experiments were carried out from 4 p.m. to 7 p.m., and during this time the animals received one subcutaneous injection of Angiotensin-II (Hipertensin CIBA) per hour. The dose of A-II was 100 μg/Kg B.W./hr (200 μg A-II/ml peanut oil). The volume drunk was measured after the test to 0.1 ml accuracy. As usually the animals themselves served as controls, after a lapse of three or four days they were injected with vehicle and the intake measured. In some cases A-II was injected first, followed by the vehicle, and in others the reverse, always following a contingent pattern.

[1] This research was done in the Department of Physiology, University of Oviedo, Spain.

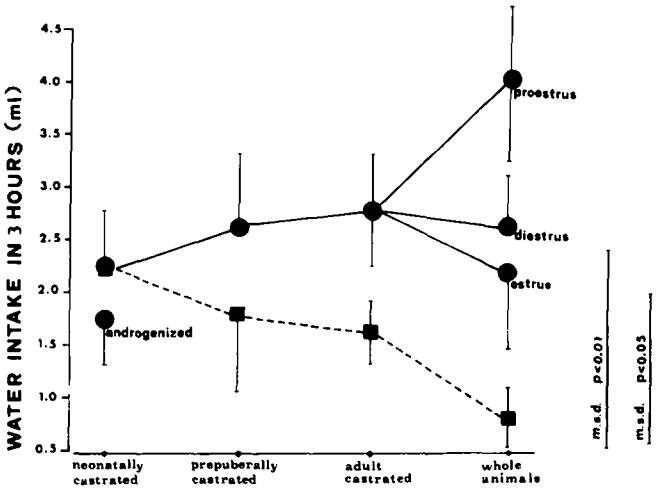

FIG. 1. Absolute volumes of Angiotensin-II-induced water intake, of male and female, in different sexual states. ●——● Females. ■- - - - - - ■ Males. m.s.d. = Minimal significant differences of means (Tukey's test), represented by vertical bars, in the right-hand side of the figure. Points are mean values of water drunk (ml) ± SE of 8–25 animals.

RESULTS

The mean value of water drunk during three hours by all males injected with vehicle (70 animals) was of 0.14 ± 0.02 ml (mean ± standard error) and that of females was of 0.13 ± 0.02 ml (97 animals); results of Student's t test (df = 165; t = 0.23) reveal no global differences of spontaneous ingestion among animals of both sexes injected with vehicle. Furthermore, the analysis of variance applied to the 11 groups of animals (males and females castrated at birth, androgenized females, males and females castrated at different stages, etc.) also shows the lack of statistically significant differences among the mean values of spontaneous ingestion for those animals, $F(10,156) = 0.87$.

The mean ingestion of males (81 cases) injected with A-II was 1.64 ± 0.21 ml, thus showing on comparing these values with those of spontaneous ingestion of animals injected with vehicle, 0.14 ± 0.02 ml, $t(149) = 56.70$; $p<0.001$, that Angiotensin-II effectively induces the males to drink.

In the females the A-II-induced water intake was 2.58 ± 0.24 ml (106 animals) which when compared to the 0.13 ± 0.02 ml (97 animals) of spontaneous ingestion of females injected with tehicle $t(201) = 100.16$; $p<0.001$) proves that A-II also shows a dipsogenic action in these animals. The analysis of variance when applied to the absolute values of water ingestion, induced by A-II in the different groups of animals of both sexes (castrated, intact, etc.), shows statistically significant differences among them, $F(10,176) = 1.974$; $p<0.05$. Minimal significant differences among the mean values of the groups (1.87 ml for $p\leq 0.01$ and 1.45 ml for $p\leq 0.05$ and represented by vertical bars on the right hand side of the Fig. 1) were calculated by Tukey's test.

A similarity in the reaction of the males and females castrated at birth and females androgenized was observed. On the contrary, when the castration is carried out in prepuberty or adulthood, there is a greater difference in the volume of water drunk after A-II administration. Finally, in the intact animals, the females in their sexual cycle show maximum water ingestion during the proestrus, statistically different from that of the estrus and the males. Also there is a significant difference between the females at diestrus and the intact males.

DISCUSSION

That Angiotensin-II administered subcutaneously induces rats to drink had already been demonstrated [6]. The relatively low volumes of water drunk by our A-II stimulated animals may be due to the low doses employed, coupled with the peripheral injection. This is the first report of sexual differences in Angiotensin-II induced thirst.

Although these differences could well be accounted for by mechanisms not directly involving the CNS, such as: a) differences in the metabolism and/or excretion of A-II, b) differences in absorption from subcutaneous fat depots, or c) differences in the ability of A-II to penetrate the BBB in the males and females, our data suggest the possible participation of nervous centres, as the preoptic areas.

From a neuroendocrinological point of view, the perinatal gonadectomy determines the differentiation of a female type of hypothalamus, independently of genetic sex [10]. Our animals castrated at birth, males and females, drink exactly the same volume, when stimulated with A-II. The androgenization with Propionate Testosterone determines a neuroendocrine differentiation of the masculine type [1]. In these animals, when adults, the A-II induced water intake, as can be seen in Fig. 1, is not statistically different from that of the other male groups, but differs from that of females at proestrus.

Finally, the differences observed in the intact animals led us to suggest the possibility that they may be mediated by a substratum related with sexual differentiation as the preoptic area, which as we know is a positive site to the A-II dipsogenic effect.

If the dipsogenic effects of Angiotensin (and one might assume other dipsogens as well) did depend on hormonal status, then by extension the effects of lesions of the basal forebrain affecting drinking would need to be interpreted with caution, since lesions in this area often change hormonal status.

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