PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 121-124 (1977)

A Pressor Response Associated with Drinking in Rats (39617)

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Intracranial injections of angiotensin II (AII) produce drinking (3, 9, 12), a blood pressure increase (5, 11), and ADH release (6, 7, 10). In our work with rats that have chronically implanted ventricular cannulae and femoral artery catheters, we have observed two components in the pressor response to intraventricular (IVT) AII, one of which is associated with the act of drinking. The presence of this drinking-dependent blood pressure increase may produce inaccurate estimations of the pressor effect of IVT AII injections if it is not taken into account in experiments where conscious rats are tested with access to water. The following experiments were designed to investigate this component of the blood pressure increase and to identify the mechanism of the response.

Methods. The subjects were 40 male Sprague–Dawley rats plus 10 spontaneously hypertensive (SH) rats of the Okamoto strain weighing 300–400 g. All animals were implanted with lateral cerebral ventricular cannulae, chronic femoral or carotid artery catheters, and in nine rats a chronic femoral vein catheter. A 3-day interval was maintained between ventricular cannulation and testing. Vascular catheters were implanted on the day of testing under ether anesthesia. For mean blood pressure recording, the arterial catheter was connected to an extended piece of PE-50 tubing which led outside the cage to a Statham P23Gb blood pressure transducer. Heart rate was measured with a Beckman 7856B cardiotachometer that was triggered by pressure pulses. Blood pressure and heart rate were recorded on a Beckman R411 dynograph recorder. For intraventricular injections, the stylus in the guide cannula was removed and a 30-gauge injection cannula was inserted. The injection cannula sat flush with the end of the guide cannula and was connected by PE-10 tubing to a 25-μl Hamilton syringe mounted outside the home cage. All animals were tested in their home cages and were unanesthetized and unrestrained during testing.

Sixteen rats received two 500-ng AII IVT tests and 12 animals were given two 50-ng AII IVT injections. All central test solutions were dissolved in 5 μl of artificial CSF: Elliott's "B," Travenol Laboratories. For one of the tests, the animal was allowed to drink water for 15 min, and in the other, water was withheld during the 15-min testing session. Rats were randomly selected to begin the first AII test either with or without water. The two tests were separated by at least 1-hr intervals.

The magnitude of the drinking-dependent pressor response was also determined in five animals before and 10 min after peripheral blockade of the alpha-adrenergic system with 10 mg/kg of phentolamine, infused iv. The stimulus used to induce drinking before and after phentolamine infusions was 50 ng of AII IVT. At the completion of testing, animals were injected IVT with 5 μl of methylene blue dye. The brains were then removed to assure ventricular access of the injections. All data are reported as mean ± SE, and comparisons were made with a paired or Student's t test.

Results. The mean resting blood pressure for the normal rats was 104 ± 2 and 160 ± 4 mm Hg for the SH rats. An example of alternate IVT angiotensin tests in one animal with and without drinking in normal rats is shown in Fig. 1. With both 500- and 50-ng AII doses, the pressor response associated with drinking resulted in greater total pressor responses than when water was withheld during testing (Fig. 2). For 500 ng of AII, the pressor response with drinking was 30 ± 3 and 20 ± 2 mm Hg without access to water ($P < 0.001$). With 50 ng of AII, the pressor response with drinking was 26 ± 2 mm Hg, and 17 ± 2 mm Hg without ($P < 0.001$). Water intake averaged 8.8 ± 0.4 ml for the 500-ng AII tests and 4.8 ± 0.7 ml for the 50-ng AII tests. In four ani-

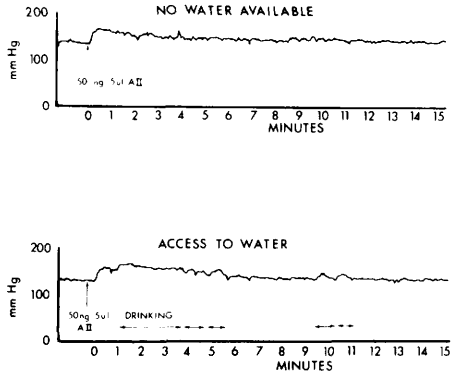

FIG. 1. Pressor responses without (top) and with (below) drinking allowed during the 15-min testing interval in Rat No. 809.

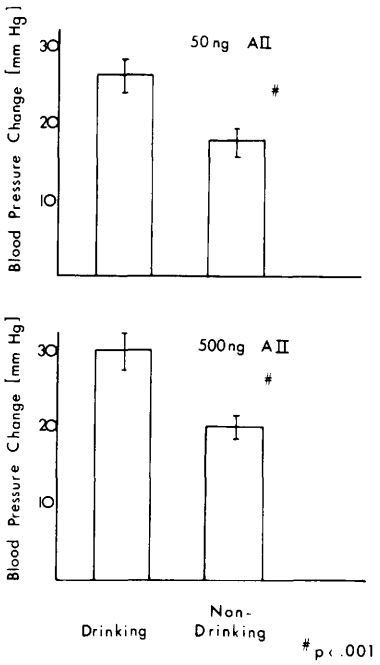

FIG. 2. Magnitude of blood pressure changes to AII IVT injections with and without drinking during the testing session; 500 ng of AII (n = 16); 50 ng of AII (n = 12). Paired Student's t test was used, drinking vs nondrinking. Values given as mean ± SE.

mals, drinking commenced after the initial pressor response had returned to baseline as in Fig. 3. In these cases, it was observed that the initial, drinking-independent response was associated with a bradycardia, while the drinking-dependent response was consistently coincident with an increase in heart

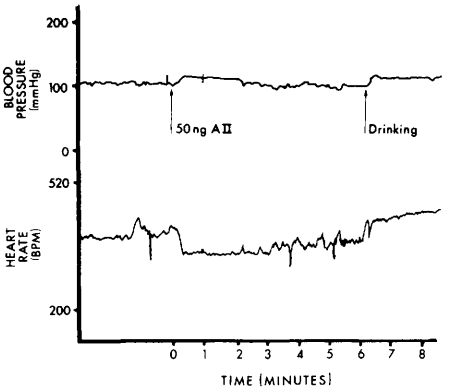

FIG. 3. Blood pressure and heart rate changes to an IVT AII test in Rat No. 1128. Initial, drinking-independent pressor response was associated with a bradycardia and drinking-dependent response was associated with a tachycardia.

rate (44 ± 8 min^{-1}). If drinking took place during the initial pressor response the additional increase in blood pressure was usually associated with no change in heart rate, although this was not quantitated. Average latencies for the pressor and drinking responses in these animals were 15 ± 2 and 70 ± 18 sec, respectively. As Fig. 1 indicates, blood pressures remained elevated during the entire drinking bout. For 10 animals tested with and without drinking to 500 ng of AII IVT, the area under the pressure curves was 225 ± 14 and 155 ± 27 mm Hg × min, respectively. This difference was significant ($P < 0.05$, paired Student's t test), indicating the pressor effect with drinking was a long-term effect. Ten animals tested with 50 ng of AII had an average pressure of 185 ± 27 mm Hg × min with drinking and 108 ± 15 mm Hg × min with no water available ($P < 0.001$). The area under the pressure curves of all animals was not evaluated because the chart speed was changed during the 15-min testing period in some cases. A pressor response during spontaneous drinking was observed in 13 rats and averaged 12 ± 3 mm Hg.

In 10 SH rats, the pressor response associated with drinking was identified separately from the initial pressor response to AII although these animals were not tested separately without access to water. The pressor response associated with drinking in

these rats averaged 25 ± 2 mm Hg. This value was greater than the drinking-dependent pressure response seen in normotensive rats ($P < 0.001$).

Four Sprague–Dawley rats were tested for drinking-dependent pressor effect to IVT AII injections while drinking from a drinking tube raised above the head, and secondly from a dish of water on the floor of the cage in order to test the effect of head position on the pressor response. There was no change in the drinking-dependent blood pressure increase between these two tests. The total pressor response with drinking from the tube averaged 28 ± 1 and 29 ± 3 mm Hg with drinking from the dish. Likewise, two animals tested in an oxygenated chamber still retained this short latency pressor response, indicated that hypoxia was not the initiating factor.

Phentolamine, an alpha-adrenergic blocker, when infused iv abolished the pressor effect of drinking in all five rats tested. Before phentolamine, the total blood pressure increase to 50 ng of AII IVT averaged 23 ± 3 mm Hg and the animals drank 4.0 ± 1.1 ml. The drinking-associated pressor response averaged 9 ± 1 mm Hg. Intravenous phentoalmine decreased the resting blood pressure in these rats from 124 ± 2 to 77 ± 4 mm Hg. After alpha-adrenergic blockade, the blood pressure increase to AII was 19 ± 3 mm Hg in these animals, and they drank 3.3 ± 0.9 ml. All animals drank in the postphentolamine-testing session. However, the pressor response associated with drinking was completely abolished by this treatment.

Discussion. The various lines of evidence we have presented here have shown the presence of a pressor response which is directly dependent on the act of drinking, and apparently independent of how that drinking is initiated. This response lasts only as long as the drinking behavior and is dependent on a functional peripheral sympathetic nervous system.

The drinking-dependent response has also been consistently observed in this laboratory with drinking induced by intracranial carbachol injections, indicating that it is not specific to AII. In addition, we have observed the response with spontaneous drinking bouts. Therefore, it appears to be a blood pressure response associated with the act of drinking, no matter how the drinking is induced.

We have not been able to define the cause of this pressor response, but we can exclude certain possible sources for it. Since no change in the two-part pressor response to IVT AII injections was seen when the animal drank out of a dish, as opposed to the drinking tube, the drinking-associated pressor response is not due to the head position during drinking, eliciting a baroreceptor reflex. The possibility that during drinking, glottis closure produces a state of hypoxia, can be dismissed. Animals which were breathing 95% oxygen in an oxygenated chamber still showed the response. In addition, the blood pressure increase is observed a very short time after the start of drinking (10 s or less), too soon for hypoxia to become apparent. Volume loading by the water intake is also unlikely to be a cause of the pressor effect because of the fast latency of its appearance. The response is not observed with eating, showing that it is specifically related to drinking. This also suggests that general muscle activity is not responsible for the effect.

No significant difference was noted in the total pressor response to IVT AII injections after iv phentolamine treatment. This is due to an activation of both sympathetic pressor mechanisms and vasopressin release with IVT AII infusions (11). Following peripheral sympathetic blockade, the sympathetic component of this response is blocked. However, the cardiovascular response to circulating vasopressin is enhanced (6). This results in a total pressor response after sympathetic blockade which is not different from the normal response (6, 11). Of primary importance to this study, however, is the fact that the pressor response associated with drinking was abolished, indicating that this particular component is of sympathetic origin. This is consistent with our observations that the blood pressure increase with drinking occurs with a short latency and falls off immediately after the drinking stops. Tachycardia associated with the drinking-dependent pressor response was consistently observed and provided another indica-

tion of increased sympathetic activity. The greater increase in drinking-related pressor responses by SH rats as compared to normotensive animals may be due to increased sensitivity of the peripheral vascular system of SH rats to norepinephrine (8) and increased vascular responsiveness of these animals to all vasoconstrictor agents (2). Increased cardiovascular responsiveness in SH rats to sympathetic activation has been noted previously (1, 4).

The results of these experiments have significance relating to experimental procedures. The phenomenon may be quite widespread over several species such as sheep (Dr. E. Blaine, personal communication) and dogs (Drs. Reid and Ganong, personal communication). Investigators dealing simultaneously with drinking and pressor responses in unanesthetized animals and manipulations which will change one or both of these should be aware of the drinking-related pressor effect.

Summary. When angiotensin II is injected into the cerebral ventricles of the rat, drinking and a short latency pressor response are produced. We have found that the pressor response has two components. The first component is always associated with angiotensin injections. The second component, however, is associated with drinking behavior. If water is not available, the second pressor component is absent. We have investigated this drinking-associated pressor response and find that it is not only seen when angiotensin is the stimulus, but also in normal or carbachol-induced drinking. The effect is not due to head position during drinking or hypoxia but it is mediated by the sympathetic nervous system since it is abolished by alpha-adrenergic blockage.

We are grateful for the technical assistance of Judy Phipps and to Dr. J. Farber for advice. This work was supported by Program Project Grant No. H107007 to W. E. H., NSF Grant No. BNS75-16364 to M. I. P. and Grant No. MRIS 7737.03 from the Veterans Administration to Phillip G. Schmid, M.D.

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Received April 6, 1976. P.S.E.B.M. 1977, Vol. 154.