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RAPID COMMUNICATION

Ouabain Injected Into the Hypothalamus Elicits Pressor Responses in Anaesthetized Rats: A Mapping Study

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JONES, D. L. AND S. LO. *Ouabain injected into the hypothalamus elicits pressor responses in anaesthetized rats: A mapping study.* PHARMACOL BIOCHEM BEHAV 36(4) 979–983, 1990.—Cardiac glycosides are known to have a narrow therapeutic index, due in part to their effects on the brain. Injections of cardiac glycosides into the ventricles of the brain elicit activation of the autonomic nervous system, and may even elicit cardiac arrhythmias. However, the specific brain regions responsible for such action are unknown. The hypothalamus receives chemo- and baro-receptive innervation from the cardiovascular system. In turn, there are both direct and indirect effector pathways from the hypothalamus accessing the sympathetic preganglionic and parasympathetic ganglia regions. This suggests that the hypothalamus may be a prime candidate for the central toxic effects of cardiac glycosides. The purpose of this experiment was to map the rostral diencephalon to determine sites at which injections of a low dose of the cardiac glycoside, ouabain, resulted in altered cardiovascular responses in the anaesthetized rat. Microinjections of 20 ng of ouabain in 200 nl were made into sites throughout the rostral diencephalon of urethane (1.2 g/kg) anaesthetized rats while monitoring heart rate and blood pressure. Injections into the nucleus medianus, paraventricular, anterior and posterior hypothalamic nuclei produced increases in pressure of from 5 to 25 mmHg. These data suggest that part of the toxicity resulting from the cardiac glycoside administration may be due to the direct action of the glycosides on these hypothalamic structures. The paraventricular region has the greatest sensitivity and may be a primary target due to its direct connections with the preganglionic sympathetic regions in the spinal cord.

Ouabain Cardiac glycosides Arrhythmia Hypothalamus Cardiovascular regulation

A classical therapy for patients with some cardiac rhythm disturbances or heart failure is administration of a cardiac glycoside, digoxin to enhance cardiac performance. The therapeutic index of digoxin is narrow due in large part to proarrhythmic effects of two subtypes: 1) A-V block and 2) ventricular tachycardia (2). Brain circuits may play a role in the A-V block (1). The origin of ventricular tachycardia is also suggested to be due to actions on the brain (2,5). However, the central site(s) responsible for this detrimental activity is unknown. The posterior hypothalamus region has been implicated in such cardiopathophysiology. Activation of the sympathetic nervous system either by electrical stimulation of the posterior hypothalamus (24) or stellate ganglia stimulation (13) predisposes the heart to arrhythmias and increases susceptibility to fibrillation (18).

Direct application of ouabain to the posterior hypothalamus of conscious cats elicited vasoconstriction accompanied by an increase in body temperature and behavioral arousal (12). Behavioral paradigms which involve limbic-hypothalamic activation also increase the toxic arrhythmogenic effect of ouabain (14). After coronary artery ligation, turnover of catecholamines in the hypothalamus is enhanced (20). Also digitalis increases metabolic activity of both the hypothalamus and the area postrema and the A2 catecholamine group, which contains the noradrenergic cell bodies that project to the hypothalamus and limbic forebrain. Since a significant component of digitalis toxicity is due to a central effect (2,21), the foregoing suggests that perhaps toxicity of digitalis may be due to activation of hypothalamic cardiovascular efferent output either via modifying afferent hypothalamic input or via directly altering efferent output due to the sensitivity of hypothalamic neurons to circulating pharmacological agents.

These results are consistent with the hypothesis that cardiac glycosides have a direct effect on arrhythmogenesis through actions on the hypothalamus to stimulate sympathetic activation. This hypothesis was tested by mapping sites in which ouabain injection elicit cardiovascular responses. A preliminary abstract of some of this work has been published (10).

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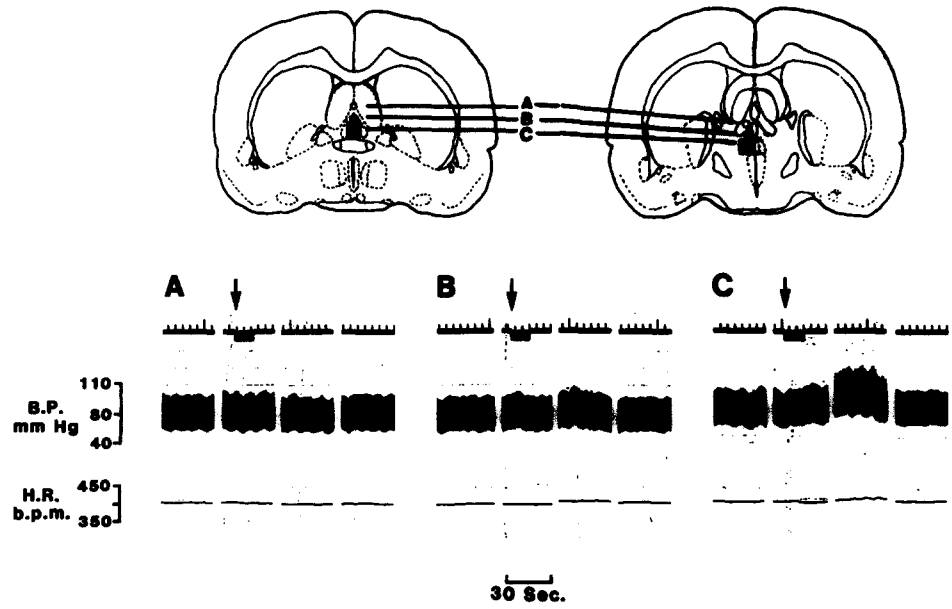

FIG. 1. Arterial blood pressure (B.P.) and heart rate (H.R.) responses in rat No. 1312 to injections of 20 ng of ouabain (arrows). There was no change in either parameter following injection into the septal area (A) or above. An increase in blood pressure of 8 mmHg and an increase in heart rate of 10 b.p.m. followed injection into the medial septal area (B). An increase in blood pressure of 24 mmHg and an increase in heart rate of 10 b.p.m. followed injection into the dorsal median preoptic and paraventricular nuclei (C). In each B.P. and H.R. record the groups of recordings are separated by 5 min. The time lines at the top of each record are 5 sec apart. Insets are at 7.4 and 6.8 mm anterior to the intra-aural line following the atlas of Pellegrino *et al.* (15).

METHOD

Male Wistar rats weighing 285–440 g were anaesthetized with urethane (1.2 g/kg), supplemented as needed throughout the experiment. The right femoral artery and vein were cannulated and the artery was connected to a pressure transducer (Statham P23id) for blood pressure recording. Output from the pressure transducer was first passed to a low level DC preamplifier (Grass model 7P1F) for blood pressure recording, the output of which was passed to an EKG tachograph preamplifier (Grass model 7P4F) for heart rate recording. Continuous records were obtained on a pen recorder [Grass model 79 (7,9)]. Using routine stereotaxic procedures (8, 9, 11), the animal was positioned in a stereotaxic frame (David Kopf Instruments) and burr holes were made in the skull above the rostral diencephalon bilaterally. Ouabain at 100 ng/μl was injected in 200 nl volumes at regions throughout the diencephalon to map sensitive sites.

Bilateral injections were made using two fixed length, 30-gauge hypodermic tubes mounted on the micromanipulator of the stereotaxic frame, connected by PE-10 tubing to two 10 μl Hamilton syringes mounted on a constant infusion pump (Harvard Instruments). Unilateral injections into the nucleus medianus, a midline structure, were made with a single injection cannula. After a stable baseline record had been obtained, mapping began with injections made at 1.0 μl/min for 12 sec. Following each injection a minimum of 10 min or sufficient time for the recordings to return to baseline, was allowed to elapse before moving the injection cannulae. Values obtained were compared to baseline values obtained immediately before injection. Injections were made in 0.5 mm steps through a depth of 3–4 mm throughout the rostral diencephalon. A minimum of three sets of injection tracks were made in each animal (9).

At the end of the experiment, the rat was sacrificed with an overdose of urethane, perfused transcardially with 100 ml buffered saline followed by 100 ml of buffered formalin. The brain was removed and immersed in formalin for a minimum of 24 hours before histological verification of injection site locations (7).

RESULTS

The minimum pressor response which was detectable was 2–3 mmHg. The minimum heart rate response which was detectable was 5 beats per min. Injections of saline throughout the hypothalamus elicited little or no discernable response. The normal preinjection mean arterial blood pressures were from 60 to 90 mmHg with heart rates from 350–490 beats/min. One of the most pronounced responses was with one injection cannula in the nucleus medianus and the other in the paraventricular nucleus (Fig. 1). The response increased at increasing depths at each of the three injection sites shown. There was also an accompanying increase in heart rate. However, for most of the sites, the heart rate response was less pronounced than the pressor response, or the heart rate response followed the pressor response, rather than occurring simultaneously with its onset. Although there were some injections into the nucleus medianus which produced responses, the responses were inconsistent with other injections in similar regions producing only weak or no discernable responses. In some sites, injection of ouabain produced either no response or the intrinsic variability was up to 5 mmHg and if there was a small response it was masked. The responses were rapid in onset, (usually less than 2 min), and the peak response was attained in 5 to 10 min. The injection sites which were most responsive were

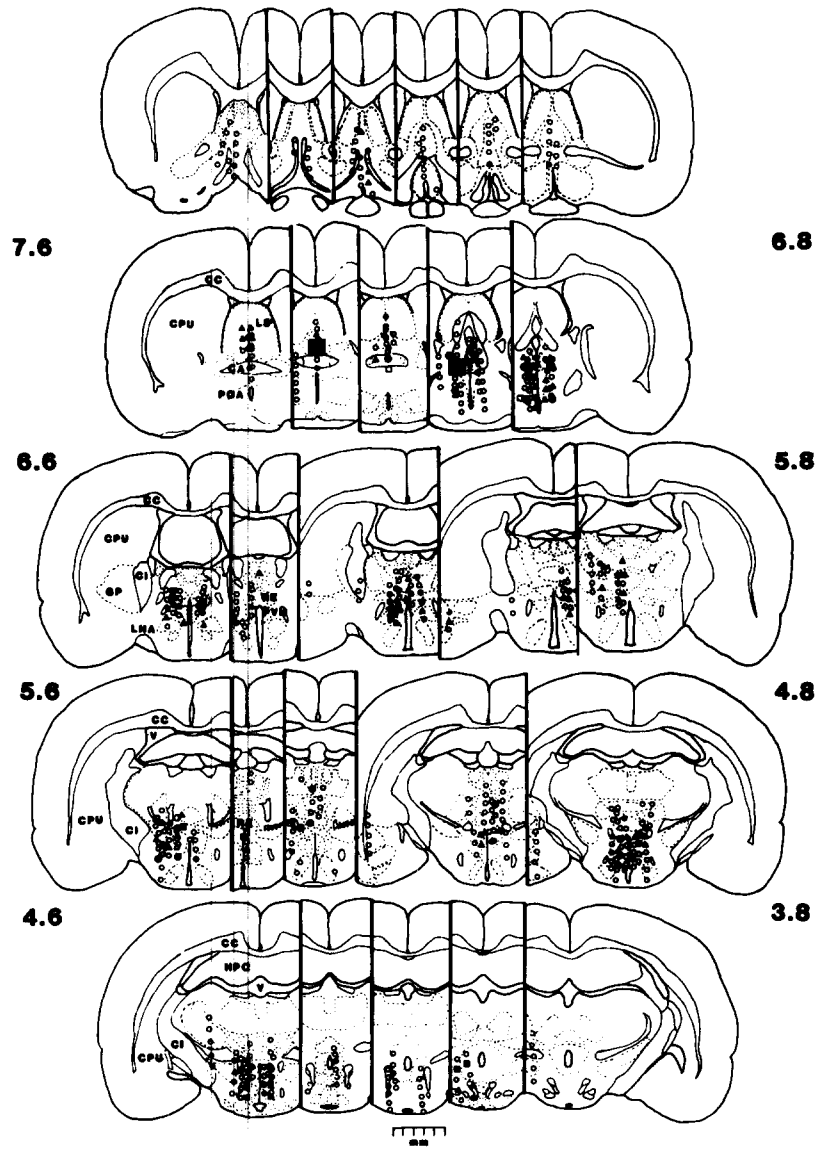

FIG. 2. Diagrammatic representation of injection sites throughout the diencephalon indicating systolic arterial pressure changes following the injection of 20 ng of ouabain. Open symbols represent changes of 0 to 5 mmHg; closed triangles 6–10 mmHg; closed circles 11–15 mmHg; small closed squares 16–20 mmHg and large closed square greater than 21 mmHg. Numbers on the left and right of each row of figures indicate the relative anterior-posterior positions of the end figures following the atlas of Pellegrino *et al.* (15).

within the hypothalamus (Fig. 2). The most responsive zones were clustered about the anterior, posterior and paraventricular hypothalamic nuclei.

Calculation of the mean responses for each nucleus indicated that the paraventricular nucleus was the most sensitive region, which also had the most consistent responses (Fig. 3). Each of the anterior and posterior regions also were responsive, the nucleus medianus was inconsistent having equalled the greatest response of any injection site and also having several sites in which there were no discernable responses. It must be recognized that the injection into this nucleus was unilateral with the other cannula in the paraventricular nucleus. There were also several sites which were outside these four prescribed regions in which ouabain injections produced responses (Fig. 3). Again, however, these responses were not consistent or the responses were weak and the sites were close to one of the four regions (anterior, posterior, paraventricular or nucleus medianus), suggesting that the response could have been due to spread of drug to one of the regions.

DISCUSSION

The main findings of this study are that ouabain injected directly into the tissue of the hypothalamus in very low doses can alter cardiovascular regulation. Direct injection into the paraven-

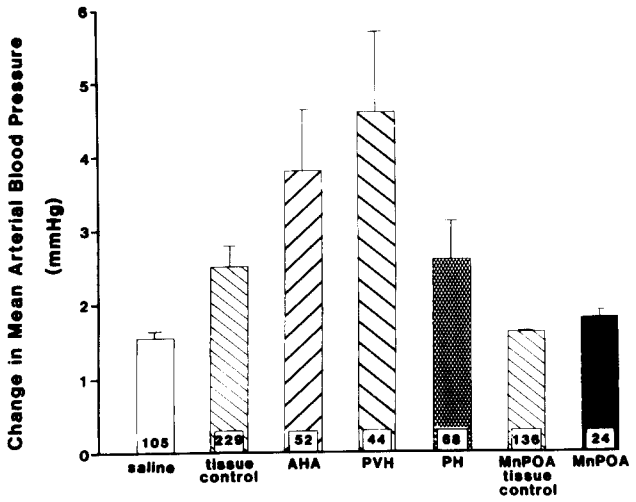

FIG. 3. Mean+SEM arterial blood pressure changes following injections of ouabain or saline control into the rostral diencephalon. For simplicity all nonresponsive sites are combined in the group called "tissue control." Numbers in each bar represent the number of injection sites in each area. Injections were bilateral except for those given into the nucleus medianus, which were unilateral. The "saline" group is for all responses following volume-matched saline injections into each of the hypothalamic sites. AHA, anterior hypothalamic area. PH, posterior hypothalamic area. PVH, paraventricular hypothalamic area. MnPOA, nucleus medianus.

tricular hypothalamus produced the most pronounced and consistent responses. These results suggest that the hypothalamus may be a prime candidate for the central toxic effects of cardiac glycosides.

In previous studies, in order to examine central sites responsible for cardiovascular effects of cardiac glycosides, relatively high doses have been administered. Injections of 80 μg/kg of ouabain into the lateral cerebral ventricle of Sprague-Dawley rats produced a pressor response within 10 min which persisted for over an hour (3). A similar dose administered intravenously was without effect. Injections of 20–300 μg of ouabain into the lateral ventricle also produced dose-dependent pressor and tachycardia responses in anaesthetized dogs and cats (17). When injections of a 0.1 to 10 μg of dihydroxyouabain were given into the third ventricle of Wistar rats, the response had a short latency (30 sec) and had peaked at approximately 5 min (23). This response indicated that structures near the walls of the anterior third ventricle in the hypothalamus were involved in the response to ouabain.

In cats anaesthetized with α-chloralose, direct injection of 10 μg in 10 μl into the ventromedial hypothalamus elicited small decreases in the heart rate and blood pressure while injections into the posterior hypothalamus elicited small increases (19). In unanaesthetized cats much lower doses (20 ng in 0.5 μl) elicited vasoconstriction and increases in body temperature. In urethane anaesthetized rats, 0.01 to 10.0 μg in 1.0 μl produced no responses when injected into the anterior or ventromedial hypothalamus although pronounced dose-dependent pressor and tachycardia responses were produced with injections into the posterior hypothalamus (6).

In the present study, using several orders of magnitude lower doses of ouabain than most previous studies, the posterior hypothalamus was also found to be sensitive to the injection of ouabain. This result is consistent with previous literature and the known anatomical link between the posterior hypothalamus and the sympathetic nervous system (4).

It is interesting that the predominant effect at this dose is an increase in blood pressure with more limited effects on heart rate. This was somewhat expected as previous investigators had indicated an enhanced sympathetic drive following ouabain administration (3, 6, 23), although contributions from neuroendocrine systems were also implicated in the cardiovascular responses (3, 17, 23). In the present study the most potent response was obtained with injections into the paraventricular nuclei of the hypothalamus. This region contains the largest number of arginine vasopressin containing cells of any nucleus in the brain (16) and has both direct and indirect connections to the spinal sympathetic preganglionic column (22). As no injection locations were shown in the previous papers in which direct tissue injections were used, and there are no comments on attempts to inject into the paraventricular hypothalamus, it is not possible to determine if there were any injections into the paraventricular hypothalamus or what the responses to such injections might have been.

One injection which was not bilateral, made into the left paraventricular nucleus and the nucleus accumbens on the midline produced a pronounced pressor and a moderate tachycardia response. Unilateral injections into the nucleus accumbens did not elicit consistent responses. The nucleus medianus sends projections to the paraventricular nucleus (22). It may be possible that the action of ouabain on the nucleus medianus is not significant by itself but may enhance the effects on the paraventricular nucleus.

A limitation of this study is that this study purposely used very low doses of ouabain and anaesthetized animals. Thus several sites which may respond in unanaesthetized animals and/or to higher doses were eliminated. Although such low doses produced pressor responses, there were less pronounced effects on heart rate. Also, with such low doses, there is no direct indication of the role of cardiac glycosides in the central involvement in arrhythmia. However, the use of very low doses is necessary in order to determine those regions which had the greatest sensitivity, and thus would be the best candidates for producing arrhythmic effects.

In conclusion, ouabain administered into the brain in very low doses alters cardiovascular function. There are pronounced differences in the sensitivity of hypothalamic nuclei to the actions of ouabain. The anterior, posterior and particularly the paraventricular nuclei have the greatest sensitivity in producing pressor and tachycardia responses to ouabain. These hypothalamic nuclei may contribute to the toxicity of cardiac glycosides.

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