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Barrel Rotation and Prostration by Vasopressin and Nicotine in the Vestibular Cerebellum

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MAITI, A., K. SHAHID SALLES, S. GRASSI AND L. G. ABOOD. *Barrel rotation and prostration by vasopressin and nicotine in the vestibular cerebellum.* PHARMACOL BIOCHEM BEHAV **25**(3) 583–588, 1986.—The aim of this study was to determine whether the primary sites for the action of vasopressin and nicotine in producing barrel rotation and prostration in rats were located in the modular cerebellum, i.e., lobule X. When arginine vasopressin was administered into either the fourth ventricles or directly into the nodular cerebellum via chronically implanted cannulae, the rats displayed intermittent barrel rotation and clonic convulsions. The administration of nicotine into the same areas resulted in prostration, atonia and, occasionally, clonic convulsions. A few days after the nodular cerebellum was lesioned with kainic acid, the motor disturbances resulting from either agent were virtually abolished. Histologic studies revealed that kainic acid had destroyed Purkinje and other large neurons, but had left the granular neurons relatively intact. The administration of procaine into either the fourth ventricles or nodular cerebellum blocked the behavioral responses of either vasopressin or nicotine given into the fourth ventricles. It was concluded that the nodular cerebellum is a primary site for the motor disturbances produced by vasopressin and nicotine.

Vasopressin Nicotine Barrel rotation Prostration Rat vestibular cerebellum Kainate lesioning

UPON administration of vasopressin [1, 5, 14], somatostatin [1,14], and chlorpromazine methoiodide [8] into the lateral or fourth ventricle, rats undergo a characteristic rotation along their longitudinal axis, a phenomenon described as "barrel rotation." The chlorpromazine methoiodide-induced response has been reported to be an antimuscarinic effect, involving the central vestibular mechanism [7,26], while the somatostatin-induced barrel rotation has been reported to be due to a direct effect on the vestibular system [9]. The barrel rotation induced by vasopressin in rats presumably involves both dopaminergic and cholinergic pathways [26].

When nicotine is administered into the lateral or fourth ventricles, rats exhibit a prostration involving all limbs and, at higher doses, clonic-tonic seizures often accompanied by barrel rotation [1]. Evidence was presented that the vestibular nuclei, particularly the lateral, was involved, but it was not clear whether the involvement was primary or secondary [2].

It is generally believed that circling behavior in rats involves mainly striopallidal and vestibular pathways [24], while a number of investigators [8,23] have proposed the existence of an important relationship between the vestibular cerebellum and the striopallidal systems. It is conceivable that peptides or chlorpromazine methoidide administered into the fourth ventricle may penetrate into the overlying nodular part of the cerebellum; a possibility which is supported by the observation that Purkinje neurones can selectively absorb small and large molecules from the cerebrospinal fluid [6]. Several cytochemical studies have indicated that the Purkinje cells, especially of the nodular area of the cerebellum, form a population that is heterogeneous chemically, morphologically, and functionally [3, 4, 13]. The present study sought to determine the effect of arginine vasopressin (AVP) and nicotine on the motor behavior of conscious rats following their administration into the nodular area of the cerebellum and to determine whether such action involves the vestibular cerebellum. Two series of experiments were carried out. In the first series, AVP or nicotine was given directly into the nodular areas of the cerebellum through chronically implanted cannulae in order to establish dose-response relationships for the spectrum of behavioral alterations. In the second series of experiments, injections of either agent were made through cannulae chronically implanted into the fourth ventricle of rats after the areas were lesioned. It was observed that the barrel rotation produced

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TABLE 1

INCIDENCE OF BARREL ROTATION AFTER INJECTION OF AVP INTO THE NODULAR CEREBELLUM AND FOURTH VENTRICLE

	Dosage of AVP and the number of animals display in barrel rotation							
	20 pmoles		50 pmoles		100 pmoles		200 pmoles	
Day	Nodular	IVth	Nodular	IVth	Nodular	IVth	Nodular	IVth
1	4/12	2/12	8/12	10/12	12/12		5/5	5/5
4th	8/12	3/12	12/12	12/12	5/5		3/3	4/4
7th	12/12	8/12	3/3	3/3	2/2*		1/1*	2/2*

*Deceased after 10 min.

Data is presented as the ratio of number of animals responding after administering 2 μl of agent into nodule or fourth ventricle (IVth).

TABLE 2

INCIDENCE OF AVP-INDUCED BARREL ROTATION AFTER KAINATE LESIONING

	Controls after KA number responding		After kainate lesioning		
Day	50 pmoles	100 pmoles	50 pmoles	100 pmoles	200 pmoles
1	6/6	6/6	0/6	1/6	2/6
4th	6/6	6/6	0/6	0/6	0/6
8th	6/6	6/6	0/6	1/6	1/6
14th	3/3	3/3	1/6	1/3	1/3

Rats were given 2 μl of AVP into the fourth ventricle 4–14 days after kainic acid.

TABLE 3

BEHAVIORAL EFFECTS OF NICOTINE ON CONTROL AND KAINATE LESIONING OF CEREBELLAR NODULE

	Control number responding	Kainate-lesioned number responding
prostration	12/12	3/12
seizures	8/12	0/12
ataxia	12/12	0/12

Rats were given 1 μl of 10^{-2} M nicotine into the fourth ventricle 2 days following the administration of 8 ng of kainic acid into the cerebellar nodule.

by either agent and the nicotine-induced prostration were abolished following kainate lesioning of the cerebellar nodule.

METHOD

Surgical Procedures

After anesthetization with chlorpentane (0.3 ml/100 g, IP, 28 Sprague-Dawley male rats weighing between 150–180 g and 27 male rats weighing between 270–388 g were immobilized in a stereotaxic frame, the occiput exposed, and a stainless steel guide cannula (o.d. 0.5 mm, i.d., 0.4 mm, length 7 mm) was positioned vertically into a hole in the skull overlying the cerebellum (coordinates: −12 mm bregma, 0.1 mm lateral to the midline [17]). Stainless steel screws were used to secure an acrylic dental cement cap to the skull in order to secure the cannula. Animals were allowed to recover at least 72 hr following surgery before testing.

Procedures for Administering Agents

AVP dissolved in saline adjusted to pH 7 with NaOH was administered in doses of 0.5–500 pmoles in a volume of 1 or 3 μl into the nodule or fourth ventricle, respectively. Nicotine in saline adjusted to pH 7 with HCl was given in doses of 5–10 nmoles into the fourth ventricle (IVC) and 2–4 nmoles into the nodular cerebellum, both in a volume of 1–2 μl. All injections were performed between 12.00 and 16.00 hr, using a 10 μl Hamilton syringe over a period of 1–5 min through the guide cannula into the nodular area. The needle was left in position for an additional minute to ensure complete delivery. Control animals received identical injections of buffered saline. The animals were then placed in a clear plastic cage and monitored for such behavioral effects as rotation, barrel rotation, running fits, and other abnormal movement.

Lesioning by Kainic Acid

The cerebellar nodule was lesioned by administering 5 ng of kainic acid in 2 μl through an internal cannula projecting 1

TABLE 4

INCIDENCE OF AVP-INDUCED BARREL ROTATION AD NICOTINE-INDUCED PROSTRATION BEFORE AND AFTER PROCAINE

Agent (given IVC)	Procaine injection into 4th ventricle		Procaine injection into nodular cerebellum	
	before procaine	after procaine	before procaine	after procaine
AVP (barrel rotation)	5/5	1/5	5/5	2/5
nicotine (prostration)	6/6	1/6	6/6	1/6

Rats were given either 100 pmoles of AVP or 10 nmoles of nicotine IVC (both in 1 μl) 2 min after 50 nmoles of procaine (2 μl) was given either IVC or into the nodular cerebellum.

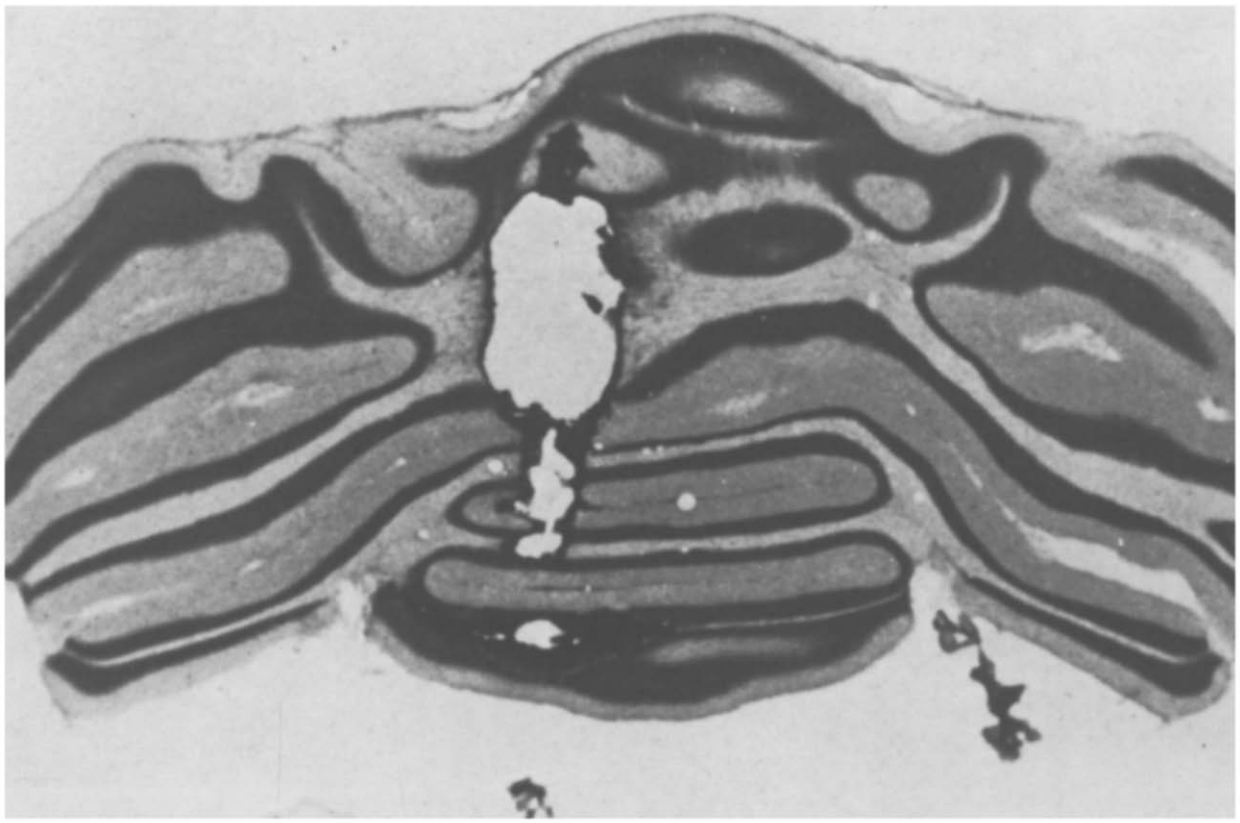

FIG. 1. Photomicrograph of section of rat brain showing cannula tract and site of dye injection at cannula tip in the cerebellar nodule. Diffusion of injected dye (2 μl of cresyl violet) seen as dark area in left half of nodule. Cresyl violet stain; magnification = 100×.

mm beyond the guide cannula. Injections were performed using a 10 μl syringe microburet, maintaining the internal cannula in place for 1 min after injection. Animals were given test agents 4–14 days after kainic acid.

Behavioral Observations

Each rat was assessed for the following responses: grooming, rearing, inactivity, running fits, circling, ataxia, backward walking, crouching, barrel rotation, and other forms of abnormal behavior. In addition, the animals receiving nicotine were evaluated for the degree of prostration and immobilization [2]. Statistics were performed using Fisher's probability test.

After each rat had received 3 injections at an interval of 3 days of either agent or saline, it was given 1–2 μl of cresyl violet or India ink into the nodular areas or the fourth ventricle, respectively, in order to determine the extent of diffusion of the solution.

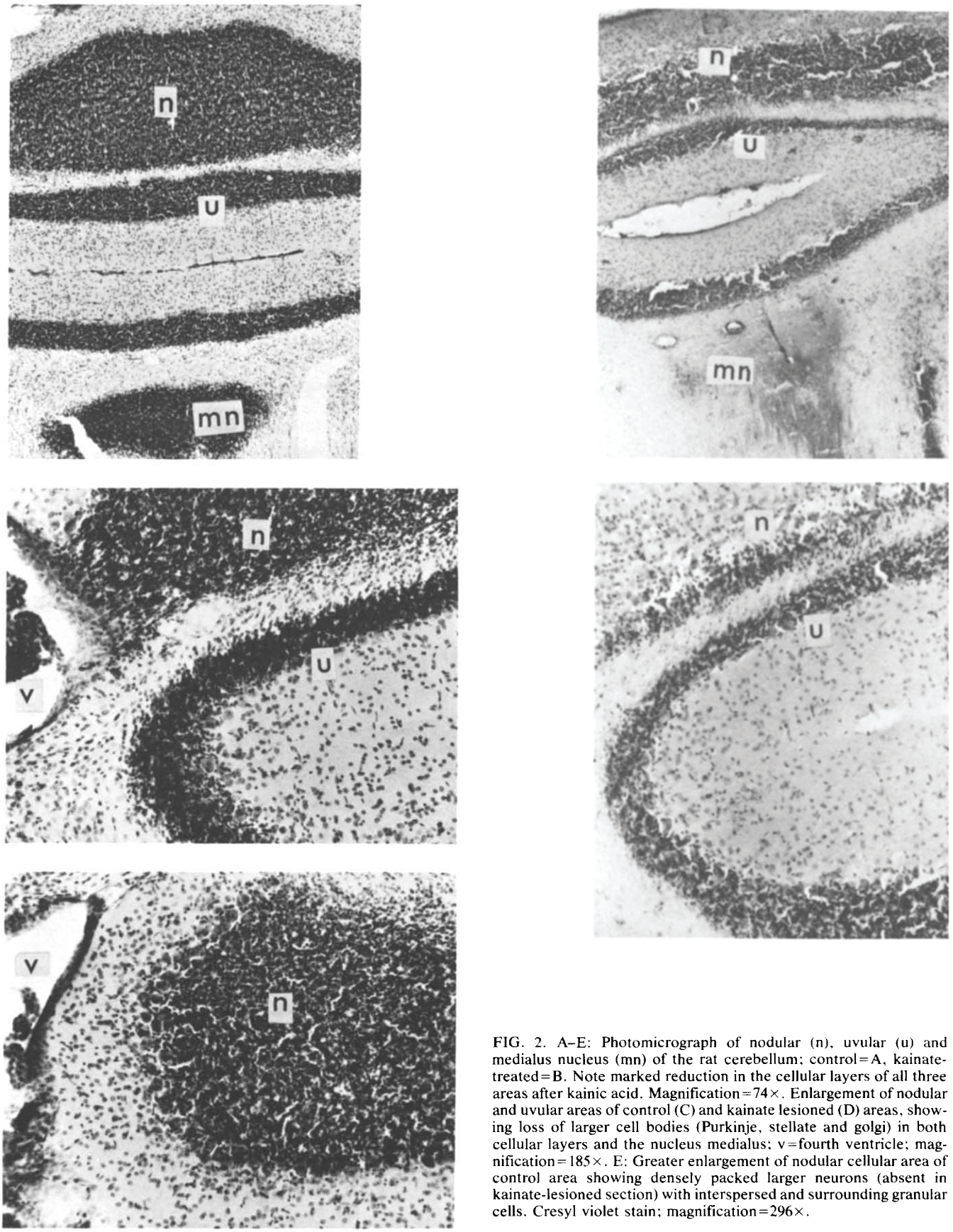

FIG. 2. A–E: Photomicrograph of nodular (n), uvular (u) and medialus nucleus (mn) of the rat cerebellum; control=A, kainate-treated=B. Note marked reduction in the cellular layers of all three areas after kainic acid. Magnification=74×. Enlargement of nodular and uvular areas of control (C) and kainate lesioned (D) areas, showing loss of larger cell bodies (Purkinje, stellate and golgi) in both cellular layers and the nucleus medialus; v=fourth ventricle; magnification=185×. E: Greater enlargement of nodular cellular area of control area showing densely packed larger neurons (absent in kainate-lesioned section) with interspersed and surrounding granular cells. Cresyl violet stain; magnification=296×.

RESULTS

Behavioral Effects of Intranodular Injections of AVP

A total of 25 normal animals from both weight groups received AVP and 18 animals received nicotine; and both drugs were given to 23 kainate-lesioned animals. Animals injected with saline showed normal movements and exploratory behavior, although the grooming behavior appeared to slightly increase immediately after injection. Injection of AVP into the nodular areas resulted in an immediate increase in locomotor activity, often accompanied by running fits. Continuous barrel rotation occurred within 1 min, lasted for 2–3 min only, and often recurred at intervals of 3–5 min with intermittent prostration or phases of exhaustion. Both clockwise and counterclockwise rotation was observed with frequent changes in the direction of rotation, followed by ataxia, body swaying (lateral head movements), and lying on one side with extended hind limbs. A total of 12 rats was given AVP intranodularly on 3 successive occasions at intervals of 3 days. At a dose of 20 pmoles AVP, 4/12 and 2/12 rats manifested barrel rotation when AVP was given into the nodule and fourth ventricles, respectively; while at a dose of 100 pmoles into either region, all animals responded (Table 1). The dose-response relationship was maintained upon successive administration of AVP. After kainate lesioning, barrel rotation was completely absent in rats receiving 50 pmoles AVP and rarely present in those receiving up to 200 pmoles (Table 2). When rotation did occur in lesioned animals, it was far less severe than in the normal animals.

Behavioral Effects of Intranodular Injection of Nicotine

Intranodular injection of 1–10 nmoles nicotine resulted in a dose-related response in motor behavior. With 1–3 nmoles nicotine, there was increased exploratory behavior with some irritability and hyperactivity, while 10 nmoles resulted in weakness, immobility and prostration. At low doses, the animals showed some rotational movements (8–12 turns per min) of only a few seconds' duration. Immediately after injection of nicotine, there was head bobbing and stereotyped sniffing activity or increased grooming. At doses of 10–20 nmoles, rats immediately displayed hyperactivity with running fits, swaying movements of the head, ataxia, and seizures of a few seconds' duration associated with clonic and tonic movements of the limbs, followed by marked prostration lasting for more than 30 min. Also noted was dystonia of the limbs, neck and body, which lasted for a few minutes. The administration of 10–20 nmoles of nicotine into the fourth ventricle led to marked prostration and flaccid paralysis of the body; while at doses of 1–5 nmoles, the animals exhibited head nodding and stereotyped movements followed by inactivity. The nicotine-induced behavioral excitation and prostration were almost completely absent following kainate lesioning of the cerebellar nodule even at higher doses of nicotine (Table 3). The initial signs of hyperirritability with a strong startle reaction and running away responses normally seen following intranodular injection of nicotine were likewise absent in kainate-lesioned rats.

Blockade of Behavioral Effects of AVP and Nicotine by Procaine

When rats were given AVP ICV 2 min after the administration of 50 nmoles of procaine HCl into either the fourth ventricles or nodular cerebellum, the incidence of barrel rotation was 1/5 or 2/5, respectively, as compared to 5/5 without procaine (Table 4). When 10 nmoles of nicotine was given ICV 2 min after procaine, the incidence of prostration was 1/6 when procaine was given by either route.

Histological Findings

Histological examination of the cerebellum taken from both normal and kainate-lesioned animals demonstrated correct placement of the cannulae (see Fig. 1) above lobule VIII and of the injection needle in the nodule of the cerebellum. The area of diffusion of the drug could be roughly marked from the spread of the injected dye at post-mortem examination; however, the diffusion of nicotine and AVP may differ from that of the dye. Histological examinations of the cerebellum taken from rats injected with kainic acid revealed areas of tissue degeneration in the uvula and nodule, characterized by widespread loss of Purkinje cells and other large cells, with little effect on granular cells (Fig. 2A–E). The observed histological appearance conformed closely to that described by others for rats with kainate lesions in other areas of the cerebellum [12,24], i.e., loss of neuronal perikarya, gliosis, and intact myelinated bundles. In 4 of 10 animals examined, the lesion involved a variable area of the nucleus medialus (Fig. 2). The damage to the cerebellar cortex resulting from the insertion of the cannula did not appear to significantly affect the response to nicotine or AVP, since lesioning with kainic acid, which completely lesioned the nodule but spared the nucleus medialus, prevented the barrel rotation or prostration observed in non-lesioned animals.

DISCUSSION

On the basis of the histological results obtained from 14 rats chronically implanted with intracerebellar cannulae, the site of the cannula tip was approximately defined by the coordinates: −11.5 to −12.5 mm of bregma, 0.1 to 0.5 lateral to the midline and 5 mm vertical from the surface of the atlas of Paxinos and Watson. Direct injection of AVP 1 mm beyond the caudal tip of the cannula (50–100 ng in 1–2 μl vol.) caused marked and consistent alterations of motor behavior. The area of diffusion of the drug appeared to be limited to 1–2 mm beyond the site of injection within the nodule, and there was little chance, if any, of leakage into the fourth ventricle. It would appear, therefore, that AVP and nicotine can induce barrel rotation and prostration, respectively, when injected directly into the nodule. Further evidence for the involvement of the nodule derives from the observation that kainate lesioning or procaine blockade of the nodule prevents the motor disturbances when either agent is administered ICV. The ability of cerebellar Purkinje cells overlying the ventricle to extract small and large molecules from the cerebrospinal fluid has recently been demonstrated [6].

Although a number of transmitters for the afferent and efferent systems of the cerebellar neurons have been investigated, the functional nature of such neurotransmitters in the vestibular cerebellum is not understood. The selective neurotoxic effect of kainic acid on glutaminergic synapses in the cerebellum has been reported by several investigators [12, 15, 16, 20, 25]. It has also been suggested that the neurotoxic action of kainic acid could also be related, in part, to its ability to increase the levels of cyclic-AMP and cyclic-GMP in rat cerebellar cortex [10,22]. It is known that kainic acid destroys all cerebellar cortical neurons except the granule cells which contain the highest density of GABA receptors in the cerebellum [12,25]. It has been reported that

the intracerebellar injection of kainate in rats markedly decreased ^{3}H-diazepam binding, while increasing ^{3}H-GABA binding [4].

The barrel rotation induced by AVP is believed to involve dopaminergic inhibition and cholinergic activation [26], and that produced by somatostatin has been shown to be blocked by atropine [8]. Conceivably, the barrel rotation induced by AVP injected into the nodule may also involve the activation of cholinergic neurons. There is considerable biochemical, histochemical, and immunohistochemical evidence that acetylcholine may function as a transmitter in the cerebellum of mammals. ^{3}H-QNB binding, along with autoradiography, have revealed the presence of muscarinic binding sites in the vestibular cerebellum [11, 18, 19], especially lobules IX, X, the part of the cerebellum receiving the primary vestibular afferents [18,19]. It has also been shown that the nodular cerebellum contains ^{3}H-nicotine binding sites [15]. Although the physiologic mechanisms underlying AVP-induced barrel rotation are not known, it is likely that muscarinic cholinergic and GABA receptors within the cerebellar nodule are involved.

The role of the vestibular cerebellum in mediating the barrel rotation is also supported by the observation that the injection of 8 ng kainic acid itself into the nodule often induced barrel rotation in rats, presumably by disrupting the vestibular-cerebellar function [15].

In conclusion, the present results suggest a role for the vestibular cerebellum in AVP-induced barrel rotation as well as the prostration produced by nicotine. The results indicate the presence of AVP and nicotine receptors in the nodular areas of the vestibular cerebellum which mediate the motor responses produced by the agents and remain intact after kainate lesioning.

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