PHYSIOLOGY

INTRACEREBRAL CONNECTIONS AFTER INJECTION OF CAINATE INTO THE CAUDAL VENTRAL MEDULLA

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Structures in the rostral and caudal parts of the ventral regions of the medulla have been shown [1, 6, 7, 10] to play a key role in monitoring activity of sympathetic preganglionic neurons of the spinal cord. Activation of neurons of the caudal part of the ventral medulla evokes sympathicolytic responses [1, 6, 7, 10], whereas their destruction causes a marked increase in perfusion pressure in vascular regions of the skeletal muscles and intestine and changes the systemic arterial pressure [2-4, 6, 10].

The aim of this investigation was to test the hypothesis [3-6] that a prolonged hypertensive response after bilateral blockade of neurons in the caudal part of the ventral medulla may be the result of disturbance of the connections of these neurons with the neurotransmitter systems in the rostral part of the ventral medulla and with neurosecretory cells of the hypothalamus involved in cardiovascular regulation.

EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats weighing 230-270 g, divided into two groups. Animals of the 1st group (23 rats), anesthetized with urethane (400 mg/kg) and pentobarbital (30 mg/kg), received an injection of kainic acid (from "Sigma," 1.25 μg in 500 nl of physiological saline, pH 7.4-7.5) into the caudal part of the ventral medulla on the right side, over a period of 5 min by means of a 1400 EC nanoliter pump (W-P Instruments-1400, USA) through a glass micropipet with a tip 50 μ in diameter, and at the rate of 100 nl/min; the 10 rats of the 2nd group received an injection of 500 nl of physiological saline. With the lambda and bregma on the same horizontal plane, the coordinates of the structures of the caudal ventral medulla were: 6.0 mm caudally to the surface marking of the lambda, 2.0 mm laterally to the mid-sagittal level, and 9.0 mm in depth from the skull surface. Blood pressure was recorded in the unanesthetized rats daily in the first half of the day in the region of the caudal artery. Changes in optical density of the surface tissues of the tail in infrared light before and after compression of the caudal artery were compared with the aid of pneumatic cuffs connected to a pressure transducer. The source of radiation was an AL-107 infrared photodiode, and the light receiver was a type FD-256 photodiode. On the 7th day after the operation the animals were decapitated, the brain was quickly removed and cooled on ice, and the basal hypothalamus, rostral part of the ventral medulla, and the caudal part of the medulla were isolated separately on the right and left sides. Sections 100 μ thick were cut on a freezing microtome from the caudal part of the medulla and stained with toluidine blue. By means of a Leitz TAS texture image analysis system (West Germany) the integral optical density was estimated in a circular mask 600 μ in diameter in the caudal part of the ventral medulla, on the left and right sides of each section alternately (Fig. 1). The intensity of neuronal uptake of neurotransmitters in the basal hypothalamus and rostral part of the ventral medulla was determined with the aid of ^{14}C- or ^{3}H-labeled biologically active

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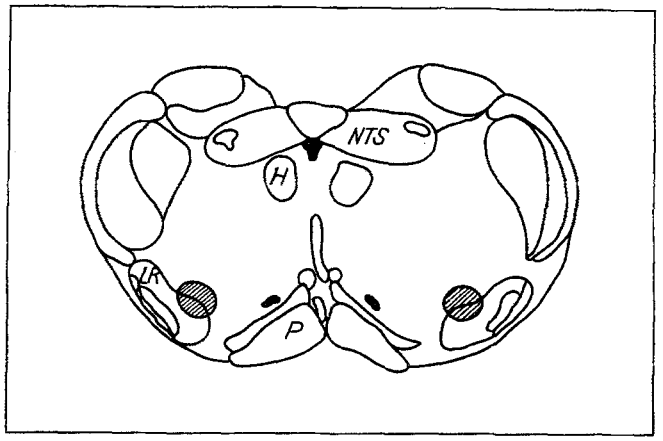

Fig. 1. Arrangement of circular masks (shaded circles) for measuring optical density in caudal part of ventral medulla. H) Nucleus of hypoglossal nerve; NTS) nucleus of tractus solitarius; P) pyramidal tract.

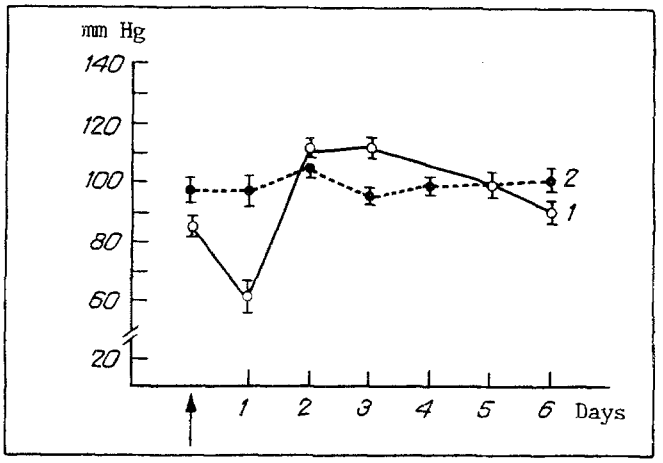

Fig. 2. Changes in blood pressure after unilateral microinjection (500 nl) of cainate (1) or physiological saline (2) into caudal part of ventral medulla. Vertical axis – pressure in caudal artery; horizontal – time of observation after operation. Arrow indicates blood pressure on day of operation.

compounds ("Amersham," England) with high specific activity: DL-5-hydroxy[G-^{3}H]tryptamine; L-[7,8-^{3}H]nonadrenalin; 4-amino-H-[U-^{14}C]butyric acid; [I-^{14}C]glycine; DL-[2,5,6-^{3}H]dopamine, and methyl-^{3}H choline chloride. A 0.1% homogenate of the corresponding tissue was prepared in calcium-free isolation medium. The intensity of neuronal uptake was determined as the difference in the number of fissions of the labeled neurotransmitters in the coarse synaptosomal fraction isolated with the aid of Sinpore No. 2 cellulose filters (Czechoslovakia) at 37°C, and in homogenates of structures of the hypothalamus and medulla kept in the cold (0-2°C). The intensity of radiation was measured on a Mark III counter (USA) and expressed as the number of counts per minute (cpm) per milligram wet weight of tissue. The experimental results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

It will be clear from Fig. 2 that 1 day after unilateral microinjection of cainate into the caudal part of the ventral medulla a significant ($p < 0.01$) fall of blood pressure by 25-30% below its initial level was observed. On the 2nd postoperative day the blood pressure showed a marked increase, and for the next 2 days it was on average 30% above the initial

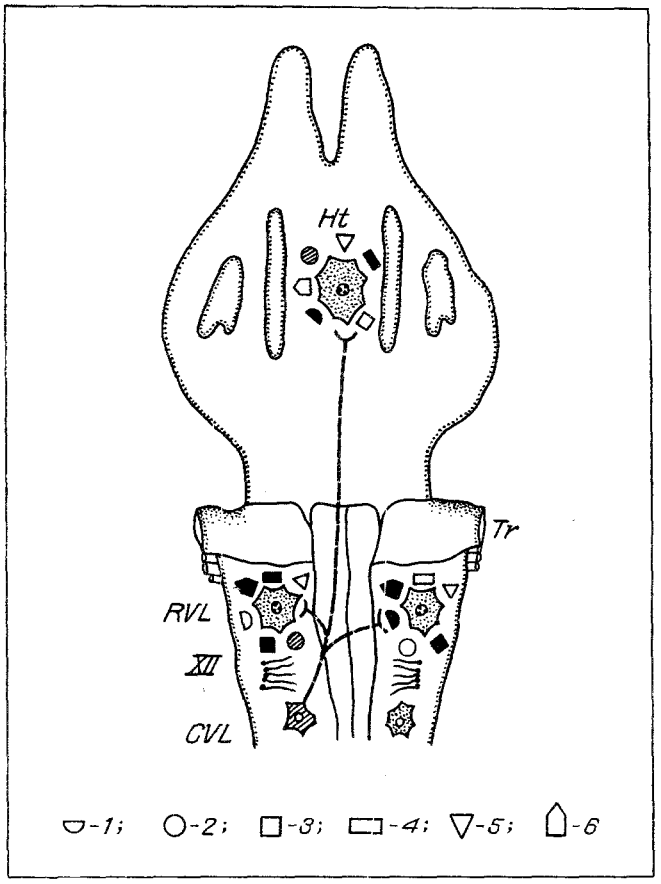

Fig. 3. Diagram showing changes in intensity of neuronal uptake of neurotransmitter in brain-stem structures after unilateral destruction of neurons in caudal part of ventral medulla. Shaded nerve cell represents zone of destruction of neurons in caudal part of ventral medulla. Broken lines between neurons indicate hypothetical connections between brain-stem structures. Neuronal uptake of neurotransmitters: 1) choline, 2) serotonin, 3) noradrenalin, 4) dopamine, 5) γ-aminobutyric acid, 6) glycine. Empty symbols denote absence, black symbols denote an increase in intensity, obliquely shaded symbols – a decrease in intensity of neuronal uptake after unilateral destruction of neurons in caudal part of ventral medulla. CVL and RVL denote caudal and rostral parts respectively of ventral medulla; Ht) basal hypothalamus, Tr) trapezoid bodies, XII) roots of hypoglossal nerves.

value ($p < 0.01$). A high blood pressure was maintained also on the 5th and 6th days, but on these days a return to values of blood pressure measured before the operation was recorded (Fig. 2).

No regular or significant changes of blood pressure were found after unilateral microinjection of physiological saline (500 nl) into the caudal part of the ventral medulla (Fig. 2).

The optical density in the zone of microinjection of cainate into the caudal part of the ventral medulla was on average 22% ($p < 0.05$) lower than in the symmetrical intact zone of the caudal part of the ventral medulla, providing objective confirmation of the visually recorded fact of neuronal destruction at the site of injection of cainate. After injection of physiological saline into the caudal part of the ventral medulla, the optical density at the site of microinjection was 0.2718 ± 0.0172 (0.2633 ± 0.0182 in the symmetrical intact zone respectively), i.e., the difference between the optical density of these parts of the brain was not significant ($p > 0.05$).

The fall of blood pressure during the first few hours after injection of cainate into the brain stem (Fig. 2) was probably connected with the stimulating action of the neurotoxin on the neuron population in the caudal part of the ventral medulla, which has a tonic inhibitory influence [6, 9, 10] on sympathetic bulbospinal neurons in the rostral part of the ventral medulla. Considering the toxic action of cainate [8] it can be assumed that after destruction of neurons at the site of microinjection, metabolic and functional changes take place initially in those structures of the CNS with which neurons in the caudal part of the ventral medulla are connected. The disturbances of regulation of the blood pressure level with predominance of a hypertensive response with effect from the 2nd day after the operation may perhaps be a result of neurochemical changes in these brain-stem formations (Fig. 2).

It will be clear from Fig. 3 that of the neurotransmitter systems investigated on the 7th day after unilateral destruction of neurons of the caudal part of the ventral medulla only the intensity of neuronal uptake of γ-aminobutyric acid remained virtually unchanged. The intensity of neuronal uptake of serotonin fell in the basal hypothalamus and rostral part of the ventral medulla on the side ipsilateral to the site of injection of cainate, on average by 40%. Cholinergic mediation increased in the basal hypothalamus and rostral part of the ventral medulla on the side contralateral to the site of injection of the neurotoxin. The intensity of neuronal uptake of glycine in the ipsilateral and contralateral structures of the rostral part of the ventral medulla increased two- to threefold, whereas the intensity of reuptake of noradrenalin in these structures increased on average by 50-55%. In structures of the rostral part of the ventral medulla on the ipsilateral side and in the basal hypothalamus, dopaminergic mediation increased (Fig. 3).

Since, of all the neurotransmitter systems studied, only the intensity of neuronal uptake of serotonin decreased in the brain-stem structures investigated after destruction of neurons in the caudal part of the ventral medulla, it must be concluded that this neurotransmitter plays a special role in the transmission of signals from neurons in the caudal part of the ventral medulla to the basal hypothalamus and rostral part of the ventral medulla. The increase in intensity of cholinergic, dopaminergic, noradrenergic, and glycinergic mediation in the structures studied in the medulla and hypothalamus probably reflects adaptation in neurotransmitter interaction aimed at restoring the initial blood pressure.

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