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Behavioral Effects of Diltiazem Injected Into the Paraventricular Nucleus of the Hypothalamus

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DE BEAUREPAIRE, R. AND W. J. FREED. *Behavioral effects of diltiazem injected into the paraventricular nucleus of the hypothalamus.* PHARMACOL BIOCHEM BEHAV **33**(3) 507–510, 1989.—The calcium channel inhibitor diltiazem is widely used as a medication for cardiovascular diseases. Some side effects have been reported after its administration, including changes in activity (apathy or hyperactivity) and feeding behavior (anorexia). Previous experiments have found that local administration of various peptides into the paraventricular nucleus of the hypothalamus can have profound effects on these two behaviors. In the present study, effects of local infusions of diltiazem into the paraventricular nucleus on locomotor activity and food intake have been tested. A marked hyperactivity, greater than the hyperactivity caused by intraperitoneal injection of amphetamine was produced. Feeding behavior was not affected one hour after the infusions but intraventricular diltiazem infusions decreased feeding behavior. It is concluded that the paraventricular nucleus of the hypothalamus has an important role in the regulation of locomotor activity and that diltiazem can act at this level to produce behavioral changes.

Diltiazam Calcium channel inhibitors Paraventricular nucleus Hypothalamus Amphetamine Locomotor activity Eating behavior

THE calcium channel inhibitor diltiazem is currently used for the treatment of cardiovascular diseases. Although the incidence of side effects with this drug appears to be relatively low, several side effects have been reported including anorexia, gastrointestinal disorders, leg oedema, dizziness, apathy, drowsiness and insomnia (4). Recently, there have been reports of increases in locomotor activity with one case of akathisia (8) and one with secondary mania (3). In previous studies we have shown that the paraventricular nucleus of the hypothalamus (PVH) and its adjacent perifornical area were important sites for the regulation of calcitonin-induced decreases in locomotor activity (2). Since diltiazem appears to occasionally decrease or increase motor activity during clinical use, we have tested the effects of diltazem application into the PVH and surrounding areas on locomotor activity. The PVH also appears to have a major role in the regulation of feeding behavior (1,9), and because anorexia is a reported side effect of diltiazem (10), we also tested the effects of application of diltiazem into the PVH on feeding behavior.

METHOD

Animals, Surgery and Infusions

Male Sprague-Dawley rats weighing 300 to 400 g were used for these experiments. They were housed in a temperature-controlled (20°C) room maintained on a 12-hour light-dark cycle. Animals were anesthetized with Chloropent (Fort Dodge Laboratories) and bilaterally implanted with chronic stainless steel guide cannulae (24 gauge, 10 mm long) terminating 2 mm above two different sites, using the following coordinates according to the Pellegrino and Cushman Atlas (13): the PVH (ant. 6.6, lat. 0.6, deep 8.0) and the lateral ventricle (ant. 5.6, lat. 2.0, deep 3.3). Testing was initiated 2 to 3 weeks after surgery. Animals were tested twice, once on each side, one time with an infusion of diltiazem (courtesy of the Laboratoires Synthélabo France) dissolved in saline, the other time with an infusion of the vehicle solution (saline) in a counterbalanced schedule. The amount of diltiazem infused was 125 μg in 0.5 μl for the PVN and 625 μg in 2.5 μl for the lateral ventricle. The volume of control vehicle solution infused was the same. The infusions were made over 60 seconds through a 33-gauge cannula connected to a Hamilton syringe. The infusion cannula was removed 30 seconds after the end of the infusion.

Procedure for Locomotor Activity Experiment

Before each infusion the animal was habituated to an open field for 30 minutes. The open field was 1 meter square with lines on the floor spaced every 0.3 m. Immediately after the infusion, testing began with the observer recording the number of lines of the open field crossed by the animal every minute over a 30-minute period. Two testing sessions (diltiazem and vehicle) were conducted for each animal separated by a one-week interval,

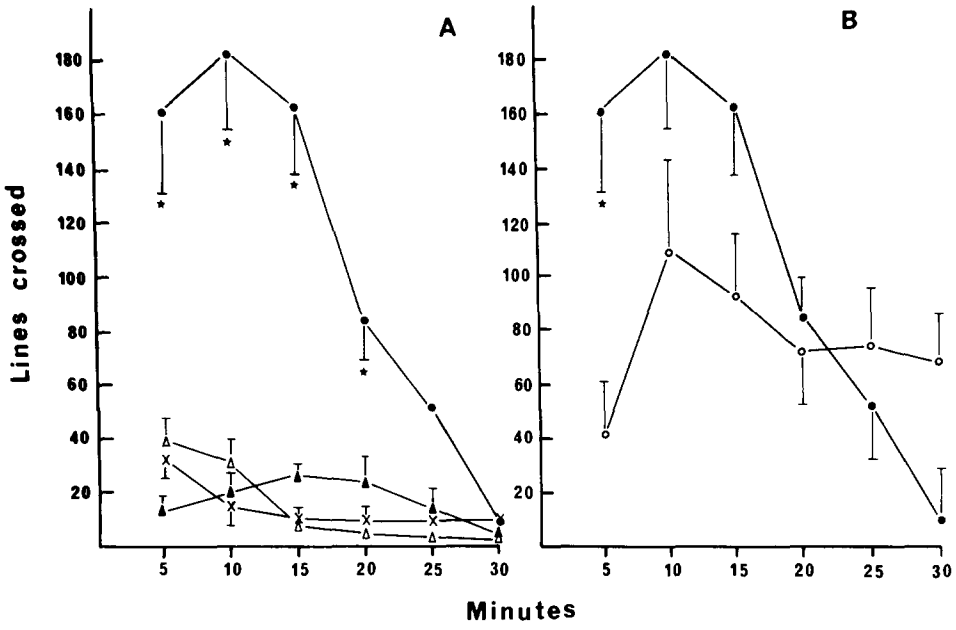

FIG. 1. Mean (±SEM) number of lines crossed as a function of time after drug administration. (A) Effects of diltiazem on locomotor activity, injections into the PVH are compared to other ways of administration. (B) Effects of intraperitoneally administered amphetamines compared to PVH infusions of diltiazem on locomotor activity. Full circles indicate PVH infusions of diltiazem (n = 10). Open triangles indicate infusions of diltiazem above the PVH (n = 10). Full triangles indicate infusions of diltiazem into the lateral ventricles (n = 10). Crosses indicate vehicle infusions (n = 20, infusions into the PVH, above the PVH and into the ventricles are combined). Open circles indicate the amphetamine-injected group (n = 8). *$p<0.01$ by Student's t-test.

always at the same hour of the day. Ten animals were tested after infusions into the lateral ventricle, and twenty-three animals were tested in the paraventricular/perifornical area or surrounding sites. Eight of these animals were randomly selected one week before the experiment, and were given an intraperitoneal injection of amphetamine 1.5 mg per kg and their activity recorded in the open field during 30 minutes.

Procedure for the Food Intake Experiment

The animals were housed in individual clear plastic cages and acclimated over 10 days to a 24-hour feeding schedule. Animals received wet mashed food (1 kg rat chow to 0.8 liter of water) for one hour per day. The mash was given in individual glass dishes at 8:30 p.m. every day and removed one hour later, so that the animals were eating in the dark. The dishes of food were weighed before and after the meal, during all habituation and testing sessions. Water was at all times available ad lib. On the day of the experiment the animals were infused at 7.30 p.m., one hour before the food was given. Six animals were tested with an injection into the paraventricular/perifornical area, and seven with intraventricular injections (each animal was tested twice, once with diltiazem and once with vehicle).

Histology

At the conclusion of testing the animals were given a 0.5 μl injection of 10% Evans Blue through the same cannula used for the drug and vehicle infusions and perfused with 10% buffered Formalin following an overdose of chloral hydrate. Brains were frozen and sectioned at 80 μm and the point of infusion verified.

RESULTS

Locomotor Activity

Infusions of diltiazem into the paraventricular nucleus or into the adjacent internal part of the perifornical area produced a very marked increase in locomotor activity (Fig. 1A). This hyperactivity started during the minute following the infusion and was immediately very marked. Some animals had periods of immobility, with cataleptic behavior for a few minutes before becoming hyperactive. The hyperactivity lasted about 15 to 20 minutes. This activity involved only running activity, with no rearing, grooming, sniffing, scratching or other abnormal behavior. After the period of hyperactivity the behavior of the animals seemed to be normal, not distinguishable from the controls, and many of the animals appeared to fall asleep. Only three of the animals did not show the typical pattern of hyperactivity. In one animal, infused into the internal part of the PVH, no hyperactivity was observed, but only a cataleptic state (immobility with occasional extension of the limbs). Another animal, infused in the anterior part of the PVH, was cataleptic for 15 minutes and then became very hyperactive. A third animal showed interspersed catalepsy and bursts of hyperactivity with little jumping movements.

Other animals, for which the diltiazem infusions were not in the paraventricular or perifornical area but in surrounding areas such as the anterior part of the fornix, the bed nucleus of the stria terminalis or the upper part of the nucleus reuniens, showed no hyperactivity. These animals had no abnormal behavior except for

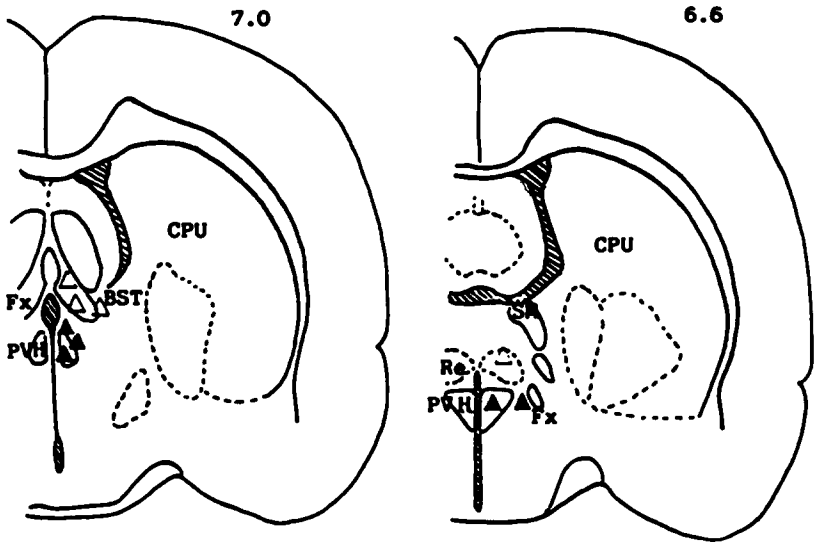

FIG. 2. Locations of diltiazem infusion sites. Full triangles indicate hyperactivity. Open triangles indicate no change in activity. BST = bed nucleus of the stria terminalis. CPU = caudate. Fx = fornix. PVH = paraventricular nucleus of the hypothalamus. Re = nucleus reuniens. SM = stria medullaris. Numbers indicate Pellegrino *et al.* (13) frontal planes.

intense tooth chattering, which was also observed in animals infused into the PVH and into the ventricles. The infusion sites are shown in Fig. 2.

The eight animals stimulated with amphetamine, randomly selected and tested before the experiment with diltiazem, had, during the first 15 minutes, an increased activity which was substantially less (but more prolonged) than the hyperactivity caused by the PVH infusions of diltiazem (Fig. 1B).

Animals infused intracerebroventricularly with diltiazem were not significantly more active than animals infused with vehicle, but all showed abnormal behavior: some animals were slightly hyperactive, others were cataleptic, and most showed long periods of catalepsy and short periods of hyperactivity. This abnormal behavior generally lasted more than 20 minutes.

Feeding Behavior

Animals infused with diltiazem into the PVH area showed no significant change in their feeding behavior (Fig. 3). Among the 6 rats injected, only one ate about 25% less than the day before, and this was an animal that had signs of catalepsy. Animals that were purely hyperactive had no change in their feeding behavior. It should, however, be noted that the food was given one hour after the infusion: Immediately after the infusion the feeding test was impossible because of the intense hyperactivity. The 7 rats infused into the ventricles ate significantly less than the controls ($p<0.01$).

DISCUSSION

Diltiazem, a calcium channel inhibitor, produces intense hyperactivity when infused into a specific site of the brain, the PVH and its adjacent perifornical area. This confirms our hypothesis that the PVH is an important site through which behavioral changes, particularly changes in locomotor activity, can be triggered. In previous studies using calcitonin (2), we found that changes in locomotor activity could be elicited from other hypothalamic and extra-hypothalamic areas. Besides the PVH and the perifornical area, the most important sites were the floor of the hypothalamus over the optic chiasma and the internal zona incerta area, but the most marked effects were obtained from the PVH. This does not necessarily mean that the PVH is the site where diltiazem can act to produce akathisia or a manic syndrome, but it is of interest that a localized central structure like the PVH is very sensitive to diltiazem and can initiate such marked behavioral

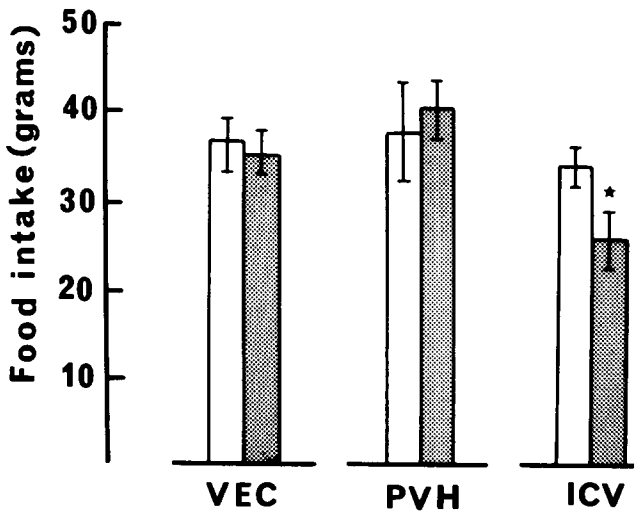

FIG. 3. Mean (± SEM) food intake the day before testing (open bars) and the day of testing (shaded bars). ICV = infusion of diltiazem into the lateral ventricle (n = 7). PVH = infusion of diltiazem into the paraventricular nucleus of the hypothalamus (n = 6). VEC = vehicle infusion (n = 9, infusions into the paraventricular nucleus of the hypothalamus and into the cerebral ventricles are combined). *$p<0.01$ by Student's *t*-test.

effects. Many animals had moments of immobility with apparent catalepsy when they were infused in the lateral ventricle, and sometimes in the PVH area. For PVH infusions signs of catalepsy appeared with infusions that were located in the anterior part of the PVH, close to the ventricles. Therefore, the catalepsy may be caused by diffusion of diltiazem into the ventricles. No diffusion into the ventricles could have occurred with more posterior PVH infusions, which were far from the ventricles and the infusion cannula tract did not traverse CSF. It is also possible that there are sites close to the PVH which are able to produce cataleptic behavior and akathisia.

Diltiazem, when infused into the PVH, did not alter feeding behavior, but intraventricular diltiazem decreased food intake. It would seem likely, therefore, that other sites in the brain are sensitive to diltiazem, and are able to produce decreases in food intake. This decrease in food intake after intraventricular infusions may also be secondary to the ability of these sites to produce cataleptic behavior, so that the anorexia would be related to periodically impaired motor activity.

The PVH is a site at which stimulation can produce several behavioral effects including changes in locomotor activity (2), feeding behavior (9), heart rate (6) and digestion (unpublished data). Side effects of diltiazem involve all of these behaviors. Although some of these side effects are probably mediated peripherally, it is possible that some side effects of diltiazem are caused by a direct action on the central nervous system, particularly the PVH. Diltiazem crosses the blood-brain barrier as demonstrated by autoradiographic studies in the rabbit (12) and the PVH is a highly vascularized structure (5). Increased calcium ion concentrations in the ventromedial region of the hypothalamus can dramatically increase feeding behavior and injections of calcium in the posterior hypothalamus raise body temperature (11). Injections of the calcium channel inhibitor verapamil in the anterior hypothalamus of the cat decrease, while injections in the posterior part of the hypothalamus increase body temperature (14). It has been suggested that this effect of verapamil is mediated by norepinephrine (15), but this may not be the case for the action of diltiazem on locomotor activity since diltiazem and verapamil differ in their ability to influence central noradrenergic neurotransmission (7).

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