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BRIEF COMMUNICATION

Stimulation of Ingestive Behaviors Following Injections of Excitatory Amino Acid Antagonists Into the Median Raphe Nucleus

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WIRTSHAFTER, D. AND R. TRIFUNOVIC. *Stimulation of ingestive behaviors following injections of excitatory amino acid antagonists into the median raphe nucleus.* PHARMACOL BIOCHEM BEHAV **30**(2) 529–533, 1988.—Anatomical and pharmacological evidence suggests that excitatory amino acids (EAA's) may function as neurotransmitters within the median raphe nucleus (MR). Previous studies have shown that injections of EAA antagonists into the MR result in marked hyperactivity. The current report extends these findings by demonstrating that intra-raphe injections of two EAA antagonists, kynurenic acid and 2-amino-5-phosphonovaleric acid, result in dose-dependent increases in food and water intake in nondeprived rats. These results suggest that EAA's within the MR may play a role in the control of appetitively motivated behaviors.

Median raphe nucleus Feeding Drinking Excitatory amino acids Kynurenic acid 2-APV Nucleus centralis superior

CONSIDERABLE evidence implicates the median raphe nucleus (MR) in the control of behavioral activation. Electrolytic or excitotoxic lesions of the MR lead to pronounced hyperactivity in a variety of situations [1, 2, 5, 12, 22, 24] and these effects do not seem to be secondary to serotonin depletion [1, 4, 6, 7, 11, 13]. Hyperactivity can also be produced by acute intra-MR injections of the GABA-A agonist muscimol [18, 25, 26] and, again, this effect does not appear to be dependent on serotonergic mechanisms [25]. The behavioral activation produced by intra-MR muscimol is not restricted to locomotor activity as these injections also result in robust increases in food and water intake in nondeprived animals [9,10]. Both the ingestive behaviors and the hyperactivity observed after injections of muscimol into the MR are much more pronounced than those seen after injections into adjacent structures such as the dorsal raphe nucleus or the ventral tegmental area [10].

Recent anatomical and neurochemical data suggest that excitatory amino acids (EAA's) may play a functional role in the MR [8, 17, 19] and we have found that pharmacological manipulations of EAA mechanisms within the MR can exert powerful effects on behavior. For example, intra-MR injections of low doses of the glutamate analogue kainic acid suppress both spontaneous and methylphenidate-induced locomotion [27]. In contrast, intra-MR injections of a number of EAA antagonists lead to hyperactivity which is similar in magnitude to that seen after muscimol injections [28]. As was the case with muscimol, injections of EAA antagonists into the MR result in larger increases in activity than do injections into the dorsal raphe or the ventral tegmental area [28]. One interpretation of these results is that a population of cells within the MR which acts to suppress behavioral activation, and which can be inhibited by GABA agonists, is subject to tonic excitation by EAA's.

The current experiment was designed to investigate whether intra-MR injections of EAA antagonists would elicit feeding and drinking in nondeprived rats. If, as we suggested above, the effects of GABA agonists and of EAA antagonists are mediated through a common population of cells, one would expect marked similarities in the behavioral responses to intra-MR injections of the two classes of compounds. Two different drugs were employed: the broad spectrum EAA antagonist kynurenic acid and the specific N-methyl-D-aspartate (NMDA) antagonist 2-amino-5-phosphonovaleric

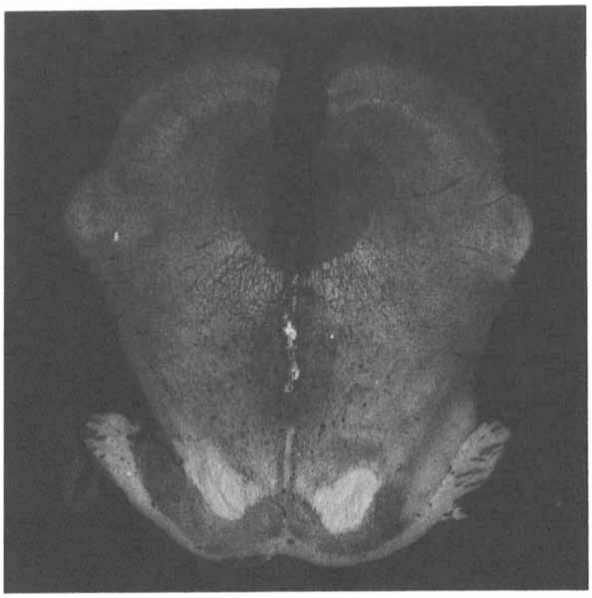

FIG. 1. Photograph of a typical cannula placement in the median raphe nucleus.

acid (2-APV) [3]. We have previously shown that both of these compounds are able to induce hyperactivity following injections into the MR [28].

METHOD

Subjects

Subjects were 23 adult, male, Sprague-Dawley-derived rats obtained from a colony maintained by the University of Illinois. Animals were maintained on a 12:12 hr light/dark cycle in individual wire mesh cages. Animals weighed between 280 and 330 g at the time of surgery. Food and water were available ad lib. Eight subjects were used to study the effects of kynurenic acid, eight the effects of 2-APV and seven were used in a control experiment to evaluate the effects of the tonicity of the infusate.

Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg). Using aseptic techniques and standard stereotoxic procedures, 22 gauge stainless steel guide cannulae aimed to terminate 2 mm above the MR (AP: −0.3; L: 0.0; H. 4.2) [16] were attached to the skull using dental cement. The cannulae were lowered in the sagittal plane following retraction of the superior sagittal sinus [23]. A 28-gauge stainless steel obdurator which extended 2 mm beyond the end of the guide cannula was then inserted.

Procedure

Rats were allowed at least ten days to recover from surgery after which each subject in the 2 APV and kynurenic acid groups was given three tests of ingestive behavior at intervals of the least three days. These subjects received injections of either kynurenic acid (0, 1.25 or 2.5 μg) or of 2-APV (0, 1 or 5 μg). Individual rats received the three injections in a randomized order. All drugs were administered in a vehicle of artificial CSF in a volume of 0.5 μl. Subjects in the control goups received, in a randomized order, injections of either CSF or CSF containing 0.73 μg of sodium chloride. The latter solution was approximately isotonic with the solution containing 5 μg of 2 APV. Injections were made at a rate of 0.25 μl/min through a 28-gauge stainless steel injector connected by polyethylene tubing to a motor driven Hamilton microsyringe. The injection cannula was removed 30 sec following the completion of the infusion at which time the obdurator was replaced and the animal returned to its home cage. While the injections were being made, a preweighed quantity of food (Wayne Lab Blox) was placed in the animals' cages and graduated drinking tubes attached to them. Additionally, sheets of paper were placed under the cages to allow for the collection of spillage. Water and food intakes (corrected for spillage) were collected one hour following injection

Histology

Following the completion of behavioral studies, rats were deeply anesthetized and perfused with saline followed by 10% formalin. Brains were stored in fixative for at least one week after which frozen, 64 μ sections were taken through the cannula tracts. The sections were then stained with cresyl violet.

RESULTS

Histological data indicated that all cannula tracts terminated within the MR. An example of a typical placement is shown in Fig. 1.

Behavioral results are shown in Figs. 2 and 3 where it can be seen that both kynurenic acid and 2-APV produced dose-dependent increases in food and water intake. The data were analyzed by means of one-way ANOVAs with repeated measures. These analyses indicated a significant effect of kynurenic acid on both food, $F(2,14)=8.02$, $p<0.01$ and water, $F(2,14)=7.88$, $p<0.01$ intake and a significant effect of 2-APV on both food, $F(2,14)=6.28$, $p<0.02$ and water, $F(2,14)=7.54$, $p<0.01$ intake. Although latencies were not formally measured, rats were frequently observed to begin eating or drinking within a minute or two after the injection. Injections of sodium chloride had no detectable effect on either food or water intake. Mean food and water intakes following injections of CSF were 1.1 g and 1.8 ml respectively, whereas the mean intakes after injections of CSF plus sodium chloride were 1.0 g and 0.8 ml ($p>0.4$).

DISCUSSION

The results of this experiment indicate that injections of 2 different EAA antagonists into the MR result in a dose-dependent increase in feeding and drinking. To our knowledge, this is the first report of alterations in ingestive behavior following central manipulation of EAA transmission. This result supports the conclusion that the behavioral effects of EAA antagonists and GABA agonists within the MR are similar and is compatible with the notion that the behavioral effects of both drugs are due to an action on a single population of cells. Given our finding that intra-MR injections of EAA antagonists produce hyperactivity [28], the current results suggest that antagonism of EAA transmission within the MR may lead to a state of "nonspecific arousal" which can manifest itself either as hyperactivity or as ingestive behavior depending on the goal objects available in the environment. We have offered a similar suggestion to account for the behavioral effects of intra-MR injections of muscimol [10]. In other studies [28] we have found that

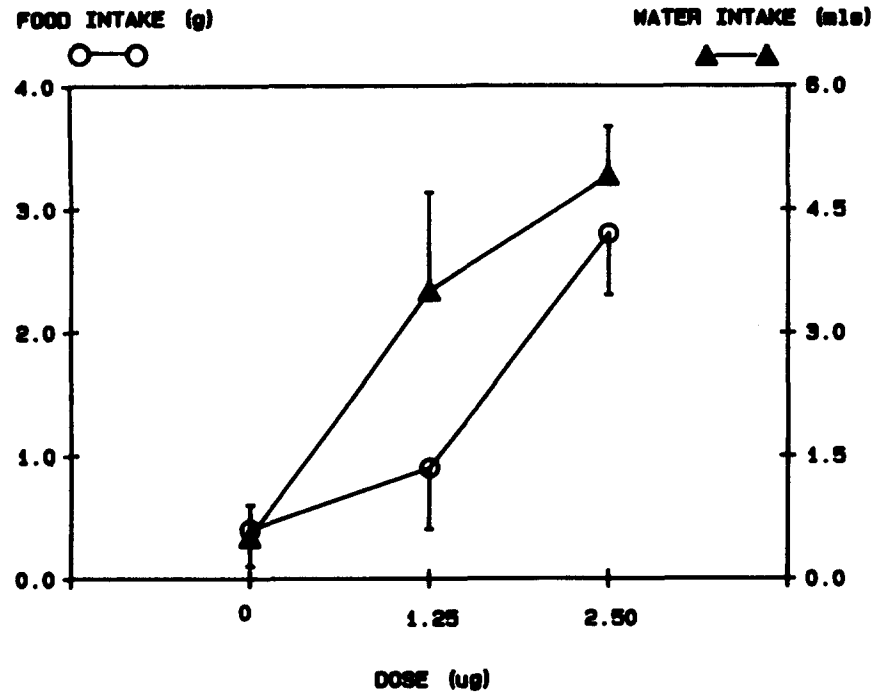

FIG. 2. Food and water intake over a one-hour period following injections of kynurenic acid into the median raphe.

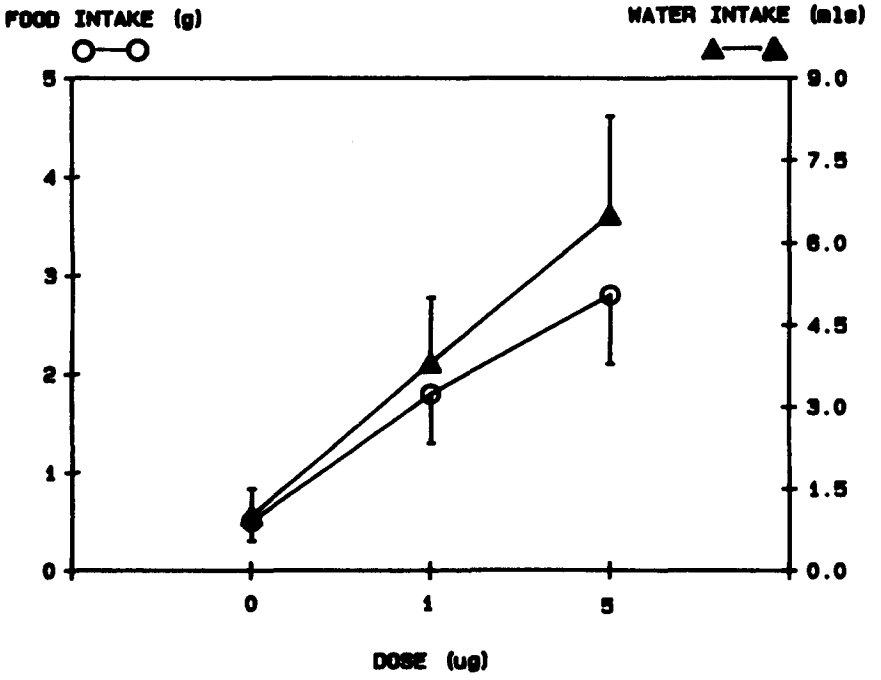

FIG. 3. Food and water intake over a one-hour period following injections of 2-amino-5-phosphonovaleric acid into the median raphe nucleus.

intra-MR injections of 2-APV lead to a decrease in serotonin turnover within the hippocampus and an increase in dopamine turnover within the nucleus accumbens, but further work will be necessary to evaluate the role of these neurochemical alterations in the behavioral effects of EAA antagonists.

The failure of the sodium chloride solution to elicit ingestive behavior suggests that the effects of the 2-APV and kynurenic acid solutions could not have been entirely secondary to their being hypertonic relative to body fluids.

It is now well-established that there exist at least three different classes of EAA receptors, the NMDA receptor, the kainate receptor, and the quisqualate receptor [20,21]. Regarding the drugs used in the current study, 2-APV appears to be a relatively specific antagonist of the NMDA receptor [3,21], whereas kynurenic acid also affects the kainate and, under some conditions, the quisulalate receptors [14,20]. In preliminary studies we have also observed increases in feeding and drinking after intra-MR injections of 100 ng of the drug CPP, which is the most potent and selective competitive NMDA antagonist currently available [21]. These results clearly suggest that NMDA receptors within the MR are involved in the control of ingestive behavior. The effects of kynurenic acid may also have been mediated through the NMDA receptor, but the possibility that antagonism at kainate or quisqualate receptors may also have played a role cannot be excluded. Unfortunately, specific kainate and quisqualate antagonists are not yet available.

The MR has been shown to receive afferents from a large number of forebrain sites which, for the most part, are related to the hypothalamus and the limbic system [15]. Given the robust behavioral effects which can be produced by pharmacological manipulations of the MR, it is tempting to speculate that descending projections to this nucleus may provide one of the pathways through which forebrain structures are able to influence goal directed behavior.

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