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Effects of Intracisternal GABA or Glutamic Acid Upon Behavioral Activity in the Rat[1]

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FREED, W. J. AND E. MICHAELIS. *Effects of intracisternal GABA or glutamic acid upon behavioral activity in the rat.* PHARMAC. BIOCHEM. BEHAV. 5(1) 11–14, 1976. – It has been postulated that GABA acts as an inhibitor of command neurons, the activity of which initiates behavior. A prediction of this hypothesis is that elevations in functional GABA levels in the brain will cause decreases in behavioral output. Accordingly, in this study rats were injected intracisternally with either saline or one micromole of GABA or its excitatory precursor, glutamic acid, and behavioral activity in a novel environment was recorded as it habituated over the course of the subsequent 4 hours. The activity of the animals that were injected with GABA was greatly decreased, while the activity of the animals that were injected with glutamic acid was apparently unaffected, as compared to animals given saline. These data provide support for the hypothesis that GABA functions as an inhibitory neurotransmitter for behavior-activating command neurons.

Gamma-aminobutyric acid Glutamic acid Amino acid neurotransmitters Intracerebral drug injections Behavior General activity Behavioral activity Locomotor activity Habituation Exploration Behavior activation Command neurons

IT HAS been postulated that behavioral sequences are initiated by spontaneously-active command neurons, the depolarization of which is normally held in check by the release of inhibitory neurotransmitter. These command neurons discharge and activate behavioral sequences when the neurons releasing inhibitory neurotransmitter are, in turn, inhibited by other neurons [28–30]. Thus, behavioral output would be under the control of a double-inhibitory system; behavior is a result of inhibition of the neurons that act to tonically inhibit command neurons.

More specifically, Roberts [29,30] has suggested that these neurons that tonically inhibit behavioral output release gamma-aminobutyric acid (GABA), while the neurons that, in turn, inhibit these gabaminergic neurons, release either dopamine or norepinephrine. A large body of experimental evidence can, in fact, be brought to bear to support this hypothesis, some of which has been reviewed by Roberts [29,30]. It is known that two areas of the brain that are intimately involved in motor control, the basal ganglia and the cerebellum, receive catacholaminergic afferents [1, 26, 34] which are usually inhibitory [16, cf. 35]. Both brain areas appear to utilize GABA extensively as a neurotransmitter [5, 10, 12, 21]. In the case of the basal ganglia, there appear to be gabaminergic interneurons [22] as well as a gabaminergic efferent pathway to the substantia nigra [11, 17, 21]. The Purkinje, basket, and stellate cells of the cerebellum are also probably GABA-releasing [6, 25, 31, 33].

Two specific predictions, regarding the regulation of behavior by neurotransmitters, can be derived from the hypothesis of Roberts [28–30]. One is that increases in catacholaminergic activity (such as are brought about by intracerebral injections of norepinephrine or dopamine agonists, for example) should cause increases in the quantity of behavioral output, because the catecholamines would be expected to inhibit gabaminergic activity, releasing command neurons from inhibition by GABA. This prediction has been verified in numerous studies [2, 13, cf. 24, 32].

The second prediction is that increases in gabaminergic activity should cause quantitative decreases in behavioral output, because GABA purportedly acts to tonically inhibit the activity of command neurons. This second prediction has not thus far been tested. Recently, however, Grimm *et al.*, [14] increased brain GABA levels using intraperitoneal injections of aminooxyacetic acid or di-N-propylacetate, and observed correlated disturbances in rats' ability to execute coordinated locomotor activities. Grimm and her co-workers ascribed these behavioral disturbances to effects upon GABA-mediated motor control processes in the cerebellum. These results are in general agreement with the hypothesis of Roberts [28–30] and, although the primary prediction in question (that the quantity of behavioral output should be decreased, as a result of elevated brain GABA levels) was not directly tested, the results indicate that further pursuit of this question is warranted.

We, therefore, attempted to provide data on the role of GABA in the regulation of behavioral output, by using direct intracerebral injections of GABA and glutamic acid, its excitatory precursor [7,18] in an investigation of

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behavioral activity in a novel environment. We chose the intracisternal route of injection because of the proximity of the cerebellum to the cisterna magna.

METHOD

Animals

Nineteen adult male Sprague-Dawley albino rats, of the Carworth CFN strain, maintained in individual wire mesh cages with free access to food and water, were used. The lights were kept on from 8:00 a.m. to 8:00 p.m. each day. These animals had been previously used for an undergraduate class in operant conditioning (cf. [23]), but no rat had previous experience in the testing apparatus.

Apparatus

A Lehigh Valley photocell activity cage was employed. This cage was circular, 60 cm in dia. and was divided into 15 cm squares by means of infrared photocell beams; each crossing of the boundary of a 15 cm square registered as one count. The floor of the cage was wire mesh, the walls were black painted metal 41.9 cm high, and the roof was black painted wood. The interior of the chamber was lit only by the light which entered through a 1.0 cm space around the bottom of the base of the cage. White noise emanated from a speaker attached to the center of the roof. After each session the cage was wiped clean and the paper under the floor was changed.

Procedure

Each animal was run once in the activity cage, starting between the hours of 12:20 and 1:40 p.m., and the cumulated activity counts were recorded for four hours, after 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min. Injections were given intracisternally, under light ether anesthesia, 2 min prior to the start of testing, in a volume of 10 microliters. Each rat was quasi-randomly assigned to one of the following three groups: (1) Control group; these animals were injected with 300 milliosmolar sodium chloride; (2) GABA group; these animals received 100 millimolar GABA (total = one micromole), adjusted to 300 milliosmolar with sodium chloride; and (3) Glutamic acid group; these animals received 100 millimolar glutamic acid (total = one micromole), adjusted to 300 milliosmolar with sodium chloride. There were seven animals in the control group, five in the GABA group, and seven in the glutamic acid group. Several additional animals exhibited a marked twisting or rolling behavior which began as soon as the animals recovered from the anesthesia and continued for several hours or even days. These animals were not run in the chambers and are not included in the above groups.

RESULTS

The mean total activity of the control group was 2323 counts in 4 hr (individual scores 3096, 1884, 2122, 1459, 3152, 1368, and 3183 counts), whereas the mean total activity of the group injected with one micromole of GABA was 561 counts in 4 hr (individual scores 556, 650, 831, 358, and 411 counts). This difference was statistically significant ($p<0.002$, two-tailed Mann-Whitney U test). The mean total activity of the group injected with one micromole of glutamic acid was 1978 counts in 4 hr (individual scores 2054, 2272, 1394, 2397, 2718, 1539, and 1470 counts); this difference from the control group was not statistically significant ($p = 0.620$).

Figure 1 shows the mean cumulative activity of each group of animals after 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min. The difference between the control and GABA groups was statistically significant at each time point (in each case, $p\leq 0.03$, two-tailed Mann-Whitney U test). However, the difference between the control and glutamic acid groups was not statistically significant at any of these points (in each case $p\geq 0.162$).

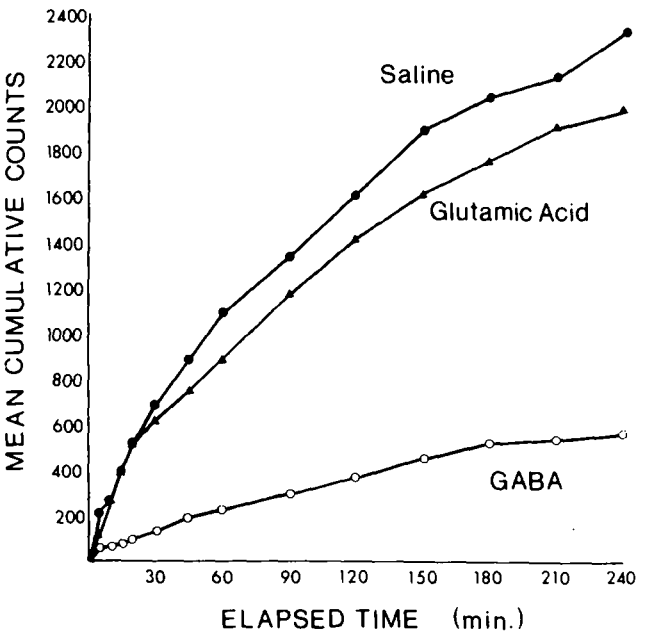

FIG. 1. Mean cumulative activity levels over the course of the four-hour session for the saline (closed circles), glutamic acid (closed triangles), and GABA (open circles) groups.

The mean activity was also evaluated for each group of animals for the first, second, third, and fourth 15 min, and for the first, second, third, and fourth 60 min periods after initiation of the session; this data is presented in Fig. 2. The difference between the saline and glutamic acid groups was not significant for any of these time periods ($p\geq 0.456$, two-tailed Mann-Whitney U test). However, the difference between the GABA and saline groups was statistically significant ($p\leq 0.03$, two-tailed) for all time periods except the third 15 min of the first hour ($p = 0.366$) and the third hour ($p = 0.268$). Inspection of Fig. 2 reveals that the mean activity of each of the three groups tended to habituate over the course of the session. Therefore, the mean activity of the GABA group remained on the order of 25% of the mean activity of the control animals for each of the eight time periods.

DISCUSSION

Intracerebral GABA caused consistent and persistent decreases in behavioral activity. The results, therefore, confirm at least one prediction of the double inhibition hypothesis of Roberts [28–30].

The results do not, at least superficially, support the findings of Mayer *et al.* [20], who reported that subconvulsive doses of thiosemicarbazide, a carbonyl trapping agent

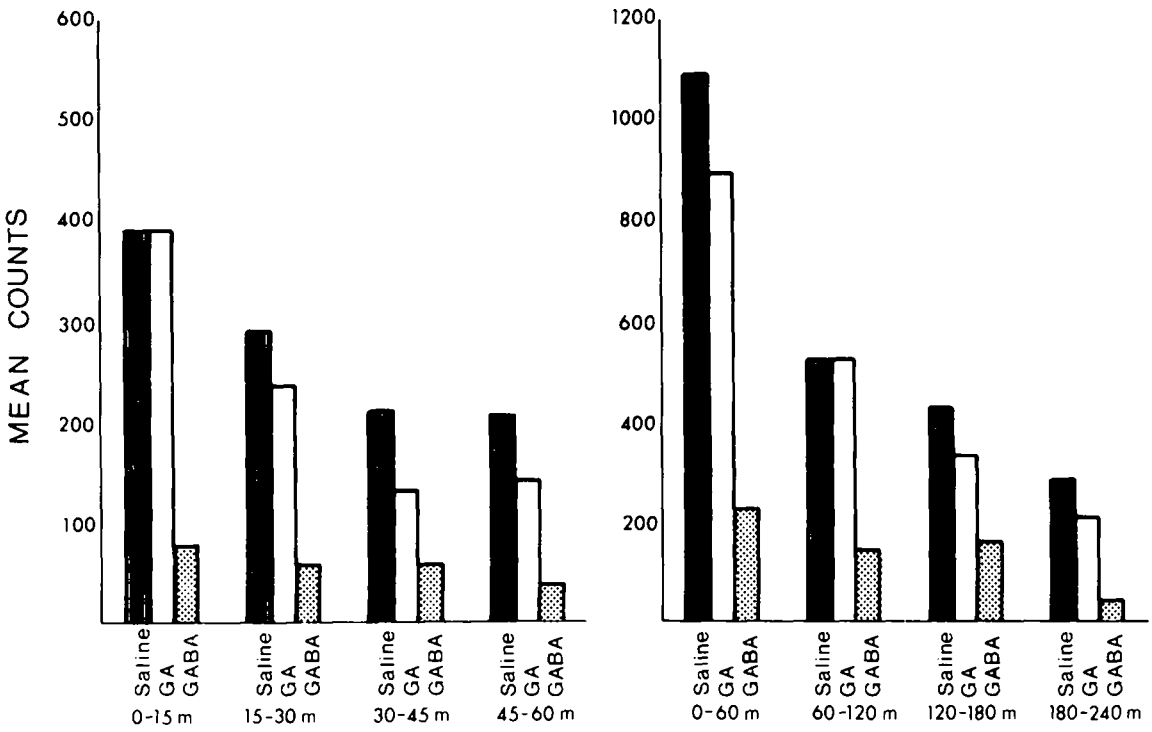

FIG. 2. Mean activity counts during the first, second, third, and fourth 15 min, and the first, second, third, and fourth 60 min, periods after initiation of the session for the saline (black bars with vertical stripes), glutamic acid (abbreviated GA; white bars), and GABA (white bars with black spots) groups.

which inhibits glutamic acid decarboxylase and decreases brain GABA levels, caused decreases in behavioral activity in a novel environment. Mayer and his colleagues attributed the activity effects to decreases in brain GABA levels; the fact that GABA itself decreased behavioral activity in the present study does not support this conclusion.

The fact that glutamic acid (the precursor of GABA) did not also cause significant decreases in behavioral activity is not surprising, in view of the fact that only a small fraction of any exogenous glutamic acid is converted to GABA, while most is converted to glutamine [3,4], an inactive compound [8,19]. However, glutamic acid may itself be an excitatory neurotransmitter [7,18], and activity changes as a direct effect of the glutamic acid might be expected. A number of studies have reported increases in behavioral activity as a result of peripheral administration of glutamic acid [9, 27, 36], and decreases as a result of peripheral injections of glutamic acid diethyl ester, a glutamic acid antagonist [9]. The fact that the blood-brain barrier is relatively impermeable to glutamic acid in the adult rat [15] suggests that these effects may not be due to a central action of glutamic acid, and the present results indicate that, in fact, they are not.

The results are consistent with the supposition that GABA inhibits the firing of command neurons, and that release from this inhibition initiates behavioral activity. Excess exogenous GABA would be expected to decrease the probability of release of command neurons from GABA-mediated inhibition and cause corresponding decreases in behavioral activity.

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