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RAPID COMMUNICATION

Effects of Harmane (1-Methyl-β-Carboline) on Neurons in the Nucleus Accumbens of the Rat

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ERGENE, E. AND E. P. SCHOENER. *Effects of harmane (1-methyl-β-carboline) on neurons in the nucleus accumbens of the rat.* PHARMACOL BIOCHEM BEHAV 44(4) 951–957, 1993.—Harmane, a β-carboline alkaloid reported to exert locomotor and psychoactive effects, is found in certain plants and also has been shown to exist in the mammalian brain as an endogenous substance. In this study, the effects of locally perfused harmane were examined on spontaneous neuronal activity in the nucleus accumbens of urethane-anesthetized rats. Extracellular single-unit recording, coupled with push–pull perfusion, enabled the discrimination of specific, dose-related effects of harmane across a wide concentration range. At lower concentrations (10^{-9}–10^{-11} M), excitation prevailed, while at higher concentrations (10^{-8}–10^{-6} M) depression was most pronounced. These findings suggest a neuromodulatory role for harmane in the forebrain reward system.

Harmane Nucleus accumbens β-carbolines Reward system Alcoholism

HARMANE (1-methyl-β-carboline) is a naturally occurring β-carboline (BC) compound first isolated in the poisonous plant *Peganum harmala* (African Rue, Mexican Rue, or Turkish Rue) (9,15). Ingestion of harmane and other BC alkaloids derived from this plant has been observed to induce profound behavioral effects in humans, including hallucinations, excitation, feelings of elation, and euphoria (7,23). At high dosage, BCs can cause a toxic syndrome characterized by tremors and convulsions (23). In animals, harmane has been reported to induce locomotor depression and hypothermia as well as tremors and convulsions (1,9,13,25,30,36). Harmane has been shown to interact with a variety of receptor systems in the mammalian brain, including those for serotonin, dopamine, and benzodiazepines (21).

β-Carbolines may be implicated in alcoholism because their concentration has been found to increase in rat brain following ethanol loading (28) and in alcoholic patients after ingestion of alcohol (31). Further, ICV infusion of harmane induces an increase in voluntary alcohol ingestion by rats (22,27). This alkaloid has been identified in the mammalian brain as an endogenous substance (3,28,29).

It is possible that central actions of harmane are mediated by the nucleus accumbens (ACB), which is an important reward circuit structure of the mammalian brain (4) and constitutes an "interface" between the limbic system and the extrapyramidal motor system (33). There is ample evidence that the ACB is involved in the behavioral actions and reinforcing properties of psychomotor stimulants (26). Recent findings support the notion that the ACB may also mediate and reinforce the alcoholic behavior (17). The purpose of this study was to examine the effects of harmane on spontaneous activity of neurons in the ACB. Preliminary results of this study have been presented in an abstract form (10).

METHOD

Thirty-nine male Sprague-Dawley rats (250–350 g body weight) were used in these experiments. Animals were anesthetized with urethane (1 g/kg, IP), cannulated with femoral arterial and venous lines, intubated to assure adequate spontaneous ventilation, and mounted in a cranial stereotaxic apparatus (David Kopf, Topanga, CA). Body temperature of the animal was maintained in the normal range (37 ± 1 °C) with a water-circulated heating blanket. Blood pressure and heart rate were recorded continuously on a polygraph to ascertain that cardiovascular function and CNS perfusion remained stable throughout the experiment. A parietofrontal craniotomy (0.5–3.5 mm lateral and 0–5 mm anterior to bregma) was

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performed, and the dura matter was reflected carefully to allow insertion of a coaxial push-pull cannula and recording electrode into the ACB. This was accomplished by placing the former at a 15° angle and the latter at a 0° angle with respect to the vertical axis. Coordinates for the ACB, adapted from Pellegrino et al.'s stereotaxic atlas of the rat brain (24), were: 1.6 mm lateral, 3.2 mm anterior to bregma, and 7.5 mm from the surface of the brain. The push-pull cannula (stainless steel, dual coaxial cannula-inlet 29 ga, outlet 24 ga) was designed and manufactured in this laboratory for perfusion of discrete brain regions, as described by others (35). With insertion of the push-pull cannula, a continuous flow of artificial cerebrospinal fluid (aCSF) was established at a rate of 25 μl/min. Composition of the ACF was: Na (143.0 mM), K (5.87 mM), Ca (2.6 mM), Mg (1.2 mM), Cl (128.2 mM), SO_4 (1.2 mM), H_2PO_4 (1.2 mM), HCO_3 (25.0 mM), and glucose (5.6 mM). It was bubbled with a gas mixture containing 95% O_2 and 5% CO_2 to adjust and maintain pH. Positive and negative head pressures for cannula inlet and outlet ports were supplied by a peristaltic pump through a pulse-dampening circuit as previously described (35).

Harmane HCl monohydrate (Aldrich Chemical Co., Milwaukee, WI) was dissolved in 0.75% acetic acid to make a stock solution of 10^{-3} M and serially diluted with aCSF to desired concentrations between 10^{-6} and 10^{-11} M. Vehicle solution for control administrations was prepared similarly, by diluting the same amount of 0.75% acetic acid solution with aCSF. The pH of all drug and vehicle solutions was between 7.0 and 7.4 at the concentration ranges employed due to the large dilution. Test or vehicle control solutions were administered for 3-min periods without any interruption of flow by switching between ports on a manifold in the perfusion circuit.

Glass-coated, platinum : iridium (70 : 30%) microelectrodes were employed to detect extracellular single-unit activity, which was monitored oscillographically. Online, count-rate analysis of neuronal discharge was performed continuously, and it was recorded on the polygraph as cumulative impulses per 10-s period. The statistical significance of changes in neuronal activity was later computed with Student's t-test, and differences between means were considered statistically significant if p was <0.05.

Upon conclusion of the experiment, the perfusion site was marked with a brief infusion of Indian ink and the recording site was lesioned by passing a direct current through the electrode. Brains were perfused transcardially with 10% buffered formalin and subsequently processed histologically in 96-μm coronal sections, stained with cresyl violet, and examined microscopically to determine the perfusion and recording sites, which were compared and referenced with the coronal sections of the rat brain from the atlas of Pellegrino et al. (24) to verify that both were in the ACB. Only those experiments in which the recording site was within 0.5 mm of the cannula tip were

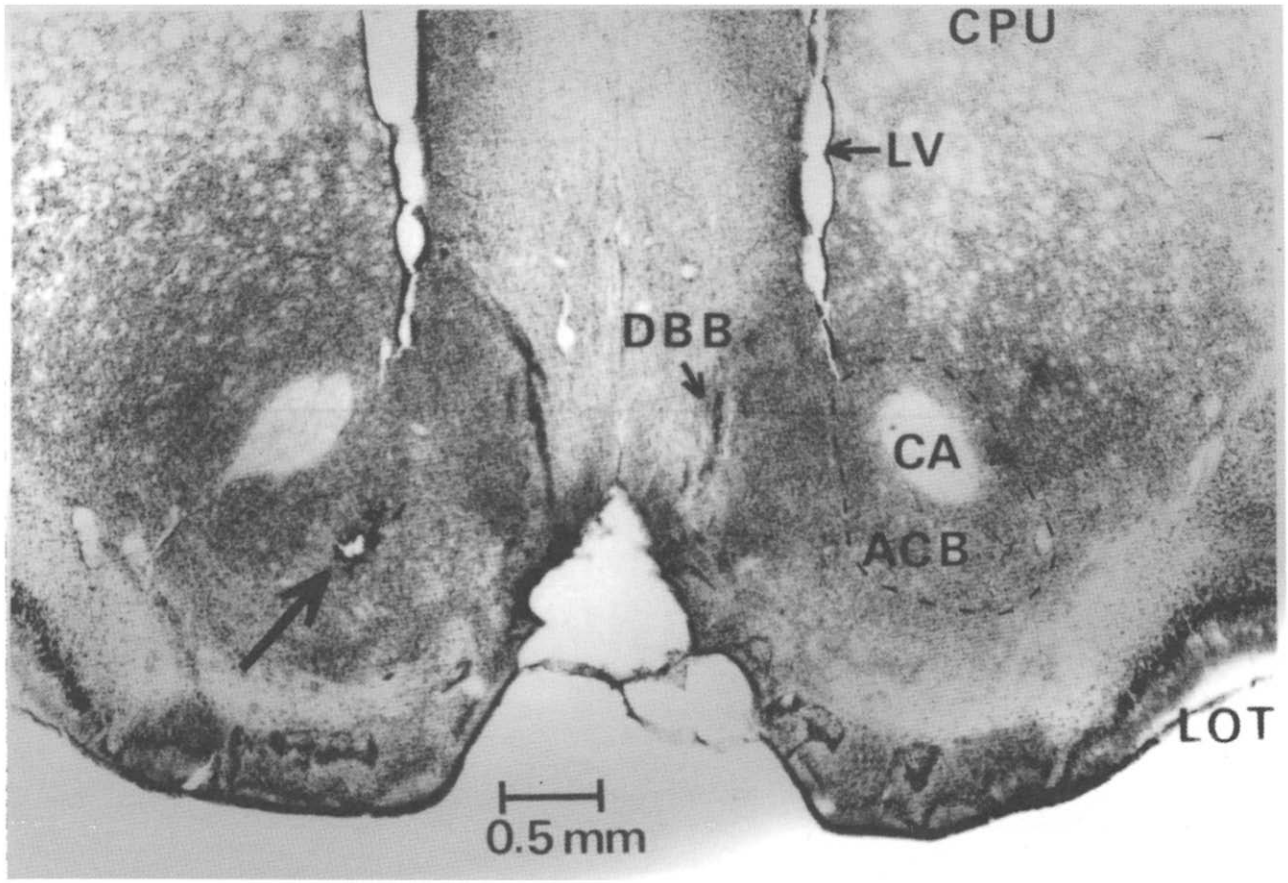

FIG. 1. Coronal section of the brain from a typical subject illustrating the location of perfusion and recording sites in the nucleus accumbens. AC, anterior commissure; DBB, diagonal band of Broca; LV, lateral ventricle; LOT, lateral olfactory tract; ACB, nucleus accumbens; CPU, caudate putamen. The arrow points to a lesion produced by current passed through the recording electrode and the stain around the lesion indicates the perfusion area.

considered for analysis. A photomicrograph of a coronal section from one of the experimental subjects shown in Fig. 1 illustrates the perfusion and recording sites in the ACB.

RESULTS

A total of 60 neurons were tested for their response to locally perfused harmane over a concentration range of 10^{-11} to 10^{-6} M. Stability of the neuronal discharge pattern prior to drug or vehicle administration was assured by pretest control observation for at least 10 min. Mean basal firing rate of the ACB neurons was 1.0 ± 0.2 impulses per sec (IPS), and most of the neurons displayed a relatively regular pattern of spontaneous activity, although a bursty, irregular discharge was observed in some cases. Three-minute control perfusions of vehicle solutions for different harmane concentrations (total n = 33) had no observable effect on neuronal activity. Unit activity was followed during the 3-min exposure to harmane and for 45 min or more thereafter to determine the rate, intensity, and duration of any change in discharge frequency or pattern that may have occurred. Recovery to the control firing rate or a new plateau was observed in all cases. Various responses were noted, including increases and decreases in discharge frequency, alteration of pattern (e.g., regular to bursty), and no apparent change in a few cases. Eighty-five percent of all the neurons tested exhibited significant change in their discharge rate after harmane; 45% of these were depressed, while 55% were excited. These effects seemed to occur with a dose-dependent relationship as summarized in Fig. 2.

Most of the neurons tested increased their discharge frequency at harmane concentrations between 10^{-11} and 10^{-9} M, while the converse was true at higher concentrations (10^{-8} to 10^{-6} M). Indeed, the plot of these nominal data revealed that doses of 10^{-10} M and 10^{-8} M were uniquely selective for excitation and depression, respectively, and that a population "null" point may exist around 10^{-9} M. Indeed, at 10^{-10} M harmane, 93% of the responsive neurons showed excitation (128 ± 29%), whereas at 10^{-8} M, 90% were inhibited (54 ± 13.3%), expressed in terms of mean ± SEM percent change from pretest control values. At the 10^{-9} M concentration, the number of units showing excitation after harmane exposure was almost equal to the number of those showing depression. These data are summarized in Table 1 and graphically illustrated in Fig. 3. Tracings of original observations are represented in Figs. 4 and 5. Typically, the mean discharge rate changed after harmane administration without modulation of the basic firing pattern as seen in Fig. 4. In some experiments, neuronal activity changed from a relatively stable baseline to a cycling pattern after harmane perfusion. Interestingly, such a change was manifested by almost half (46%) of the neurons excited at the 10^{-10} M concentration of harmane. The average period of these fluctuations was 11.4 min/cycle. It may be noteworthy that both the time to onset and duration of effect were shorter for inhibitory responses than for excitatory responses (see Table 1).

DISCUSSION

This is the first demonstration of harmane action on individual ACB neurons with local administration in vivo. It reveals a characteristic dose-dependent, bimodal response. Pre-

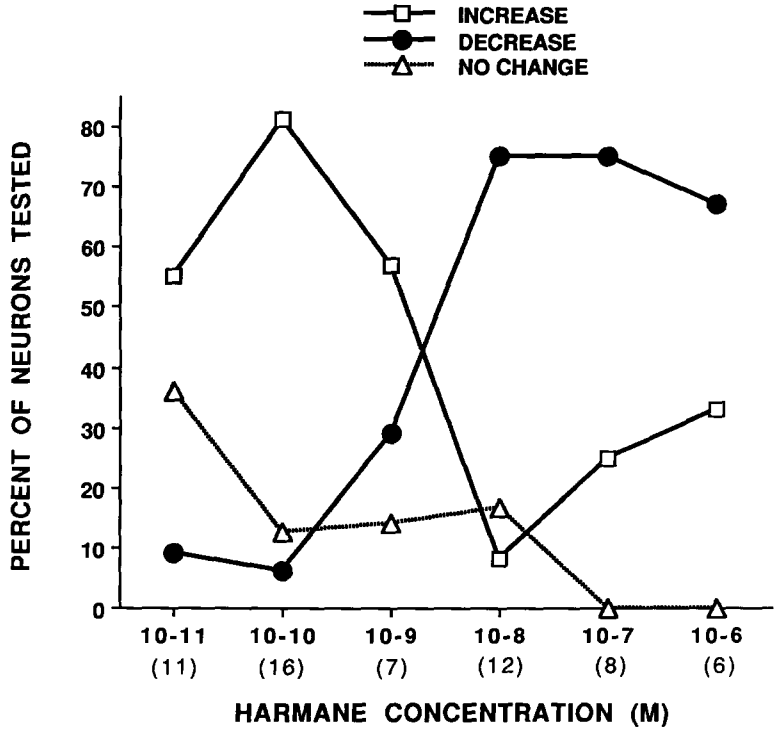

FIG. 2. Summary of the excitation and depression observed after push-pull-perfusion of harmane on nucleus accumbens neurons as a function of concentration. Total numbers of neurons tested for each dose of harmane are indicated in parentheses.

TABLE 1

EXCITATORY AND INHIBITORY EFFECTS ON NEURONS IN THE NUCLEUS ACCUMBENS ELICITED BY HARMANE AS A FUNCTION OF DOSE

Concentration (M)	Trials (n)	Discharge Rate Change (IPS)	Time to Onset (min)	Time to Peak (min)	Duration (min)
10^{-11}	6	0.95 ± 0.38	8.9 ± 1.0	20.2 ± 4.5	33.7 ± 8.5
10^{-10}	13	0.72 ± 0.19	4.7 ± 1.0	20.1 ± 4.6	29.5 ± 4.9
10^{-9}	4	0.72 ± 0.40	8.1 ± 2.8	22.3 ± 12.1	28.8 ± 12.4
10^{-8}	9	−0.36 ± 0.10	3.3 ± 0.9	9.0 ± 2.0	26.2 ± 6.5
10^{-7}	6	−0.31 ± 0.10	2.8 ± 0.9	13.2 ± 3.2	20.3 ± 4.4
10^{-6}	4	−0.38 ± 0.10	2.0 ± 0.5	10.0 ± 1.9	20.0 ± 6.5

Values are presented as mean ± SEM. All values for change in discharge rate were significantly different from vehicle administration at $p < 0.05$.

vious behavioral studies have documented a number of diverse effects for harmane, such as motor depression, ataxia, catatony, convulsions (1,9,13,25,30), and reinforcement of alcohol consumption in rats (22,27). Although experimental studies in humans are lacking, ingestion of harmala alkaloids reportedly produces hallucinations, excitation, feelings of elation, and euphoria (7,23).

Modulation of ACB neurons by harmane is particularly noteworthy from a physiological/behavioral perspective. The ACB is an important element of the forebrain reward system in which information from limbic structures, particularly the amygdala and hippocampus, gains access to the motor system (33). The nucleus thus plays a major role in the translation of motivation into action. The ACB has been implicated in spontaneous exploratory behavior of mammals, appetitive motivation, and reward processes (4). Behavioral actions and reinforcing properties of drugs abused by humans, including opioids and psychomotor stimulants, are also thought to be mediated by the ACB (8,26).

Although there is no specific evidence regarding harmane's endogenous levels in the ACB, this BC has been identified in almost all brain regions examined in the rat. It is distributed widely in brain tissue at concentrations in the low nmol/g range (3,28,32).

The response of ACB neurons to harmane exposure displayed a bimodal pattern in the present study. The robust, selective excitatory responses elicited by low concentrations of harmane indicate a specific, direct action on these neurons. Other mechanisms might come into play at higher concentrations, where the principal response was inhibition. In fact,

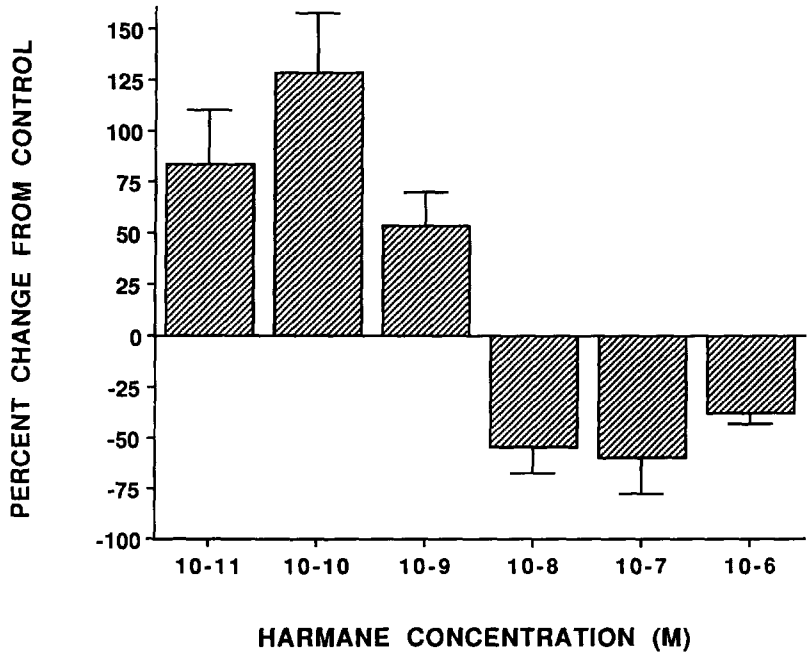

FIG. 3. Mean (±SEM) peak increases and decreases of neuronal discharge rate as percent change from control. Values represent aggregated data of dominant responses at each dose. All values were significantly different from control at $p < 0.05$ (Student's t-test).

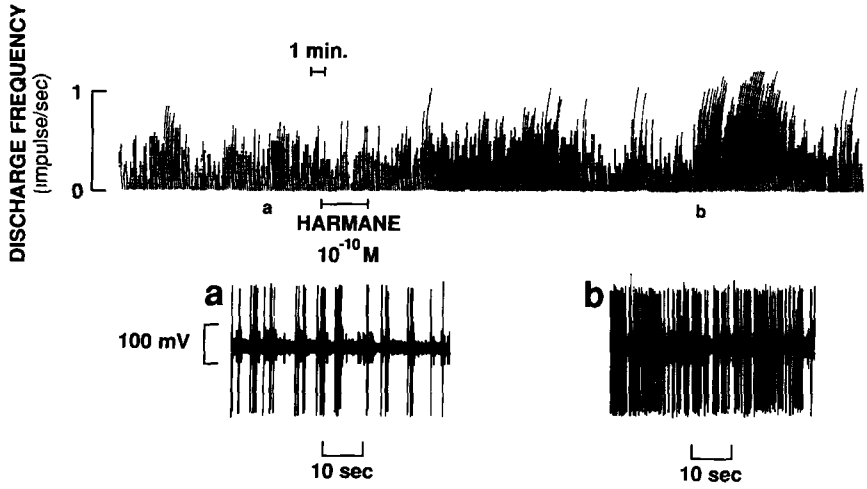

FIG. 4. Example of an excitatory response following perfusion of harmane at 10^{-10} M. The upper tracing is a continuous record of average neuronal discharge rate (10-s epochs). Below are oscilloscope tracings of the neuronal activity observed at points a and b in the rate record.

harmane has been reported to interact at different concentrations with a variety of receptor systems in the mammalian brain, including those for dopamine (DA), serotonin, and benzodiazepines (BDZs) (21). All these transmitters have been shown to play important roles in the ACB mechanisms of behavioral control (5,12,18). For example, harmane reportedly interacts with dopaminergic receptors, as shown by its inhibition of [^{3}H]spiroperidol binding to calf striatum (IC_{50} = 163 μM) and inhibition of apomorphine-induced licking movements in rats (21). Brose et al. (5) recently reported that a synthetic BC analog of harmane, *N*-methyl-β-carboline-3-carboxylate, modulates release and turnover of dopamine in the ACB of the rat.

The ACB receives serotonergic projections from the medial and dorsal raphe nuclei and appears to be involved in certain serotonin-mediated locomotor behaviors, such as lateral head-weaving (18). Brain serotonin levels (6,21) are known to be elevated by BCs, which are also capable of binding to and stimulating 5-hydroxytryptamine (5-HT) receptors directly (1, 21,34). IC_{50} values for inhibition of [^{3}H]serotonin binding by harmane have been measured in the low micromolar range (21). Therefore, it is conceivable that harmane may also mod-

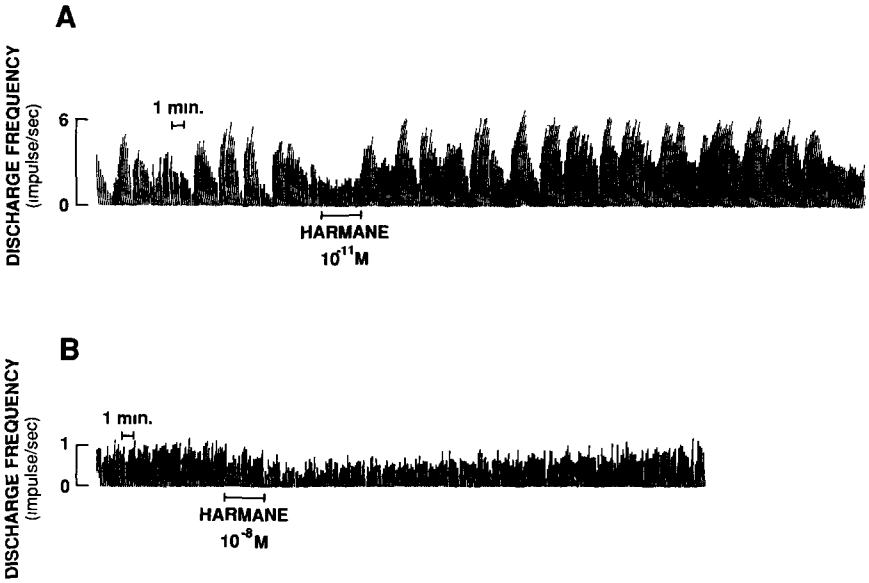

FIG. 5. Examples of neuronal responses in two different experiments. Excitation was evident in A following perfusion with 10^{-11} M harmane, while depression occurred in B after perfusion of the drug at 10^{-8} M.

ulate serotonergic transmission in the ACB. In fact, many reported behavioral effects of harmane, including tremors, psychostimulation, and ataxia, are similar to those observed in the so-called "serotonin syndrome" elicited by serotonin agonists (16).

On the other hand, several BCs have also been shown to bind with high affinity to BDZ receptors (14,30). It has been proposed that an endogenous BC ligand for BDZ recognition sites might be released by stress to interact with such recognition sites (2). Harmane may be a good candidate for this ligand. In fact, harmane does seem to be capable of binding to BDZ receptors, inhibiting [^{3}H]-flunitrazepam binding with IC_{50} values of around 7 μM in rat whole-brain homogenates (30). Further, harmane-induced convulsions can be inhibited competitively by diazepam (30). Interestingly, Mhatre and Ticku (20) have recently shown an upregulation of BDZ binding sites and a selective increase in binding of a BC inverse agonist for these binding sites in cultured neurons chronically treated with ethanol. From these observations, they proposed that an increase in concentration of an endogenous ligand for BDZ receptors might cause these changes and thereby produce ethanol tolerance in alcoholism (20). This hypothesis is supported by studies showing that the formation of harmane in the rat brain increases following ethanol loading (28) and in alcoholic patients after ingestion of alcohol (31). Further, ICV administration of harmane has been observed to reinforce alcohol ingestion by rats (22,27). It is noteworthy that BDZs also have reinforcing properties, purportedly mediated by the forebrain reward system (12). The BDZergic system also appears to interact functionally with the dopaminergic system in the ACB. For example, BDZs decrease extracellular concentration of DA (12) and block stress-induced increases in DA turnover in this nucleus (11). Harmane may be involved in these interactions at the ACB level of behavioral control because it exerts effects on both systems.

In conclusion, the present data reveal that harmane can induce specific, sensitive, and dose-related changes in the activity of ACB neurons. They suggest that this BC may be an endogenous modulator of neuronal function in the reward system. Molecular mechanisms of this action and its implications in limbic system/reward circuit-mediated behaviors warrant further attention.

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