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Tetrahydro-β-Carboline Micro-Injected Into the Hippocampus Induces an Anxiety-Like State in the Rat

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HUTTUNEN, P. AND R. D. MYERS. *Tetrahydro-β-carboline micro-injected into the hippocampus induces an anxiety-like state in the rat.* PHARMACOL BIOCHEM BEHAV 24(6) 1733–1738, 1986.—Guide cannulae for bilateral micro-injection were implanted stereotaxically in the rat to rest just dorsal to the hippocampus. Following recovery, 1,2,3,4-tetrahydro-β-carboline (THBC) hydrochloride in a concentration of 10 or 50 ng was infused bilaterally into the animal's hippocampus in a volume of 3.0 μl. In the control condition, the artificial cerebrospinal fluid (CSF) vehicle was micro-injected into the hippocampus and a sham injection was made prior to the CSF or THBC infusion. The behavioral response of the rat was examined subsequently in an open-field chamber, in terms of the number of grid squares crossed, duration of grooming time and instances of freezing-immobilization during the test interval of 7.5 min. Other behaviors recorded included the appearance of tail rigidity and the number of fecal boluses excreted. The intra-hippocampal infusion of the 10 ng dose of β-carboline reduced the motor activity of the rat whereas the higher dose of THBC increased the duration of the freezing-immobilization. THBC failed to alter significantly the grooming activity of rats or their rate of defecation. Following repeated micro-injections of 50 ng of THBC, the duration of freezing-immobilization gradually decreased, but the response itself remained essentially intact. These results suggest that the well-known anxiogenic action of certain of the β-carboline class of aldehyde adducts may be mediated in part by neurons in the hippocampus, or the constituent pathways of this limbic system structure, or both.

Tetrahydro-β-carboline (THBC) Hippocampus Anxiety Fear-like behavior Emotional responses
Open-field behavior Indole-aldehyde adduct Limbic system Micro-injection of THBC

RECENT studies have demonstrated that the ester derivatives of β-carboline-3-carboxylic acid can bind to benzodiazepine receptor sites [3, 6, 19, 25, 31]. In so doing, these compounds may alter the anxiolytic and anticonvulsant actions of benzodiazepines [6, 30, 33]. Under appropriate experimental conditions, they can induce an effect which is opposite to that produced by the benzodiazepines [11], an anxiogenic or fear-like state [12, 26, 30]. In studies assessing behavioral changes following the peripheral administration of a β-carboline, symptoms most consistently displayed in rats include tremor associated with a state of extreme fear or anxiety [5,33], freezing-immobilization and lateral head weaving [29,33]. The intraventricular (ICV) injection of a β-carboline in the rat has also been found to induce epiliptiform activity, tail rigidity, ataxia, hyperactivity, and vocalization to touch [29].

Over and above the induction of an anxiety-like state, a β-carboline infused chronically or acutely into the animal's cerebral ventricle also can produce an abnormally high volitional intake of ethyl alcohol solutions in the rat [4, 23, 35]. In a series of experiments carried out to localize anatomically certain of these effects, we found initially that 1,2,3,4-tetrahydro-β-carboline (THBC) infused directly into the hippocampus of the rat causes an abrupt cessation of its activity. The purpose of this study, therefore, was to characterize the effect of this β-carboline on the hippocampus in terms of quantitating the anxiety-like behavior exhibited by the rat in an open-field chamber [10]. These responses included freezing-immobilization, motor activity, grooming behavior, and colonic motility as reflected by fecal expulsion.

METHOD

Male Sprague-Dawley rats (n=16) weighing 450–680 g were housed individually at an ambient temperature of 21–23°C and kept on a 12-hour light cycle. Food and water were freely available to the rat and its body weight was measured at the same time each day.

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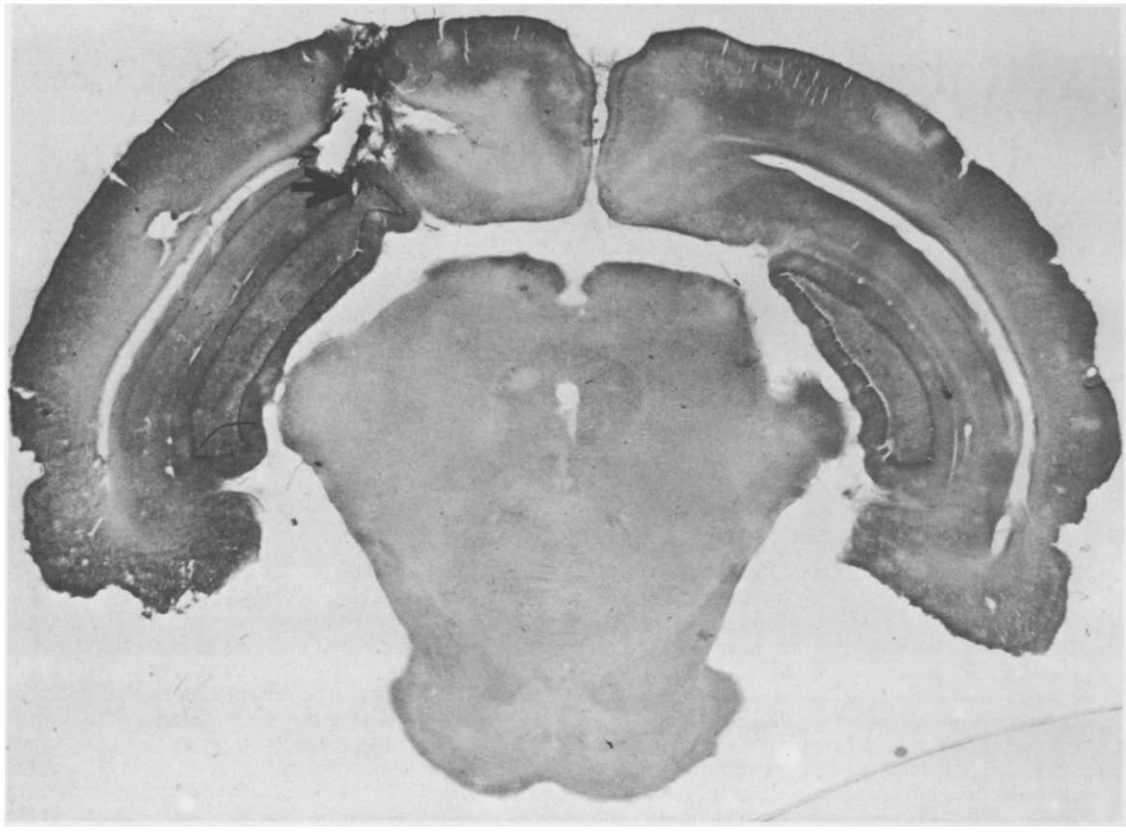

FIG. 1. Representative histological section, depicting site of THBC micro-injection (arrow), stained with cresyl violet (magnification ×10).

TABLE 1

DURATION OF FREEZING-IMMOBILIZATION DURING TEST INTERVAL OF 7.5 MIN IN OPEN FIELD*

Group	Treatment	Duration of Freezing-Immobilization (sec)
I (N=10)	CSF	18 ± 27
II (N=10)	10 ng of THBC	61 ± 69
III (N=10)	50 ng of THBC	67 ± 45
IV (N=6)	50 ng of THBC	118 ± 70

Values expressed as mean ± S.D.

*Rats in group III were treated with CSF and 10 ng THBC prior to 50 ng dose of THBC. Rats in group IV were treated initially with 50 ng THBC after sham injection.

Surgery

A 23-gauge thin-walled stainless steel guide cannula, 13 mm in length, was stereotaxically implanted bilaterally in the hippocampus of 16 rats according to surgical procedures described previously [21]. The cannulae were positioned at an angle of 7° according to the following coordinates [27]: AP, 1.0–2.5; L, 4.0; and HV, 2.5 below the dura.

Micro-Injection of β-Carboline

THBC (Sigma) was micro-injected into the hippocampus in doses of 10 ng and 50 ng given bilaterally. An artificial cerebrospinal fluid (CSF) containing Na^+ 127.6 mM, K^+ 2.5 mM, Ca^{++} 1.3 mM, Mg^{++} 1.0 mM and Cl^- 134.5 mM [21] was passed-through a 0.22 μm Millipore filter and used as the control as well as carrier vehicle. Each injection was made 1.0–2.5 mm below the tip of the guide cannula, by means of a Harvard Model 935 infusion pump, in a volume of 3.0 μl, over an interval of 3.0 min. This volume and slow infusion rate assured uniform penetration and spherical distribution of the drug within the brain's parenchyme [22,23]. To verify this, the spread of the micro-injected fluid was examined further by infusing 3.0 μl of 10% methylene blue dye into the hippocampus of three additional rats. Histological sections cut at 20 μm were then stained with hemotoxylin. Dye was observed in the rostral-caudal aspect in 20–22 sections, denoting a spread of up to 0.5 mm, with a similar penetration in the other dimensions. Little if any solution effluxed into the lateral cerebral ventricle.

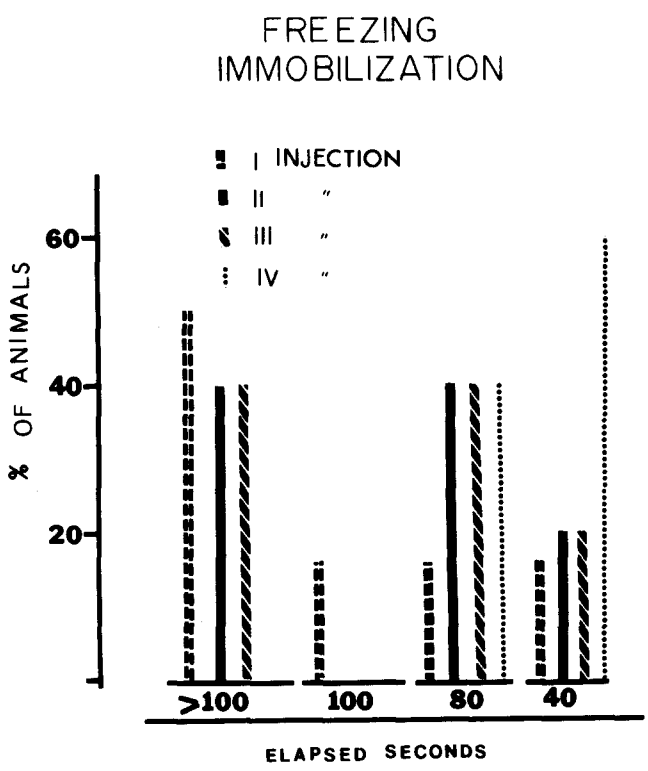

FIG. 2. Effect of 50 ng THBC on freezing-immobilization of rats re-tested repeatedly following four micro-injections of this β-carboline (N=6).

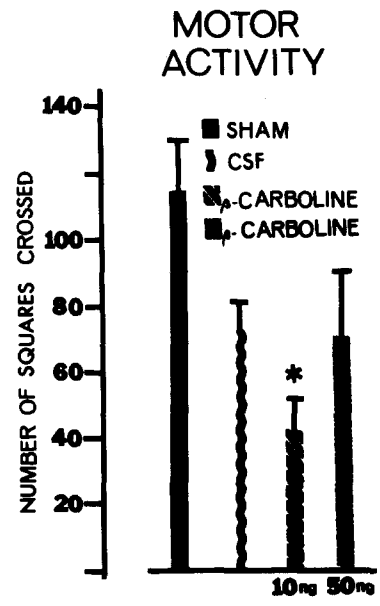

FIG. 3. Motor activity measured by number of squares crossed (±S.E.) in open-field test during test interval of 7.5 min. *Denotes significance of $p = <0.05$ compared to CSF-injection (N=10).

Prior to a set of micro-injections, a sham injection was always made by removing and replacing the indwelling guide tube stylet; thereafter, the rats were habituated to the open-field environment. The CSF solution was micro-injected into the hippocampus of five of the rats following a micro-injection of 10 ng of THBC. In five other rats, the 10 ng THBC-injection was made before the CSF-treatment. A period of 5 hours elapsed between each of the injections. On the following day, the 50 ng dose of β-carboline was injected into the hippocampus of the same 10 rats. To eliminate a possible effect due to repeated injections, the 50 ng dose of THBC was also micro-injected into the hippocampus of six additional rats which had not as yet been examined. In subsequent tests, the action of the higher dose of THBC was tested repeatedly by injecting the 50 ng of THBC at the same sites four times in those rats (n=6) treated initially with 50 ng of THBC. A period of 24 hr elapsed between each of these latter injections.

Open-Field Testing

The open field consisted of a sound-attenuated wooden chamber measuring 76×76 cm and divided into 36 squares measuring 12.7 cm per side. The open-field behavior of the rat following the intrahippocampal micro-injection of THBC as well as a sham injection was examined for a standard test period of 7.5 min [12]. Two observers were used to determine observed reliability which was always greater than 0.90 for all measures.

Records were taken of the number of squares crossed, as well as the interval of time during which grooming activity and the state of freezing-immobilization occurred. The latter response was characterized by a sudden cessation of motor movement, whole body shivering after the immobilization phase, as well as a statue-like posture [5]. The time limit of the recorded reaction was in 5 sec blocks. Other observed responses also recorded were sniffing, whisker twitching, tail rigidity, and the number of feces deposited during the 7.5 min test interval. At the end of each test, the walls and the floor of the chamber were wiped clean.

Histological and Statistical Analysis

Each rat was given an overdose of sodium pentobarbital injected intraperitoneally. The animal was perfused by cardiac puncture with 0.9% saline followed by 10% buffered formalin solution. The brain was blocked, sectioned on a cryostat in the coronal plane, and serial sections mounted and stained with cresyl violet using standard histological procedures [37]. Verification of the locus of each cannula and micro-injection site was performed using light microscopy. Figure 1 depicts a representative site denoted by the arrow.

Statistical analyses were performed using two-tailed t-tests. The responses to 10 ng and 50 ng of THBC were compared to those of the CSF-control tests in the same animals (paired t-test), whereas the effects of 50 ng of THBC injected initially were compared to those of the CSF-group

TABLE 2

THE EFFECT OF β-CARBOLINE ON MEAN ± S.D. GROOMING TIME IN SECONDS

Treatment	Time (sec)
CSF	39 ± 34 (N=10)
β-Carboline (10 ng)	77 ± 72 (N=10)
β-Carboline (50 ng)	58 ± 41 (N= 6)

*See legend of Table 1.

(unpaired t-test). In the latter case, the anatomical sites were not identical but the effect of repeated injections of the β-carboline at the same site was eliminated.

RESULTS

The bilateral micro-injection of THBC into the hippocampus of the rat reduced the motor activity of the animal in terms of both a decrease in square crossings in the open field and an increase in the time of freezing-immobilization. The dose of the indole-aldehyde adduct injected affected the specific motor responses differentially. The responses of each of the rats were not differentiated on the basis of a specific site of action of THBC. Further, no evidence of a significant degree of tolerance was found on repeated injections at the same site. Vocalizaton to touch, crawling, biting and tail rigidity and, in 30% of the rats, epiliptiform activity was observed when rats were re-tested on the higher dose of THBC. However, the β-carboline in either dose did not alter the rate of defecation as measured by the number of excreted fecal boluses in the open field.

Freezing-Immobilization

Table 1 shows that following the injection of CSF-control solution into the hippocampus (n=10), a very short-lived freezing-immobilization occurred in 50% of the animals with a mean duration of 18±27 sec. Although the 10 ng dose of THBC (n=10) induced a freezing-immobilization of greater than 100 sec in 40% of the rats, the effect on the mean duration of the reaction of 61±69 sec was not significantly different from that of the CSF-injection, $t(9)=2.09$; N.S. However, the duration of freezing-immobilization induced by the 50 ng dose of THBC (n=10) differed significantly, $t(9)=2.96$; $p<0.05$, from that of the CSF-control duration.

In all of the rats (n=6) given initially the 50 ng dose of the β-carboline in the hippocampus, the freezing-immobilization response of greater than 100 sec occurred in nearly 70% of the animals. The mean duration of 118±70 sec was significantly different, $t(14)=4.11$; $p<0.01$, from the reaction of the CSF-group. No significant difference was found in the duration of the response (Table 1) between the two groups given the 50 ng dose of THBC, $t(14)=1.77$.

Although the number of animals exhibiting a persistent freezing-immobilization declined, as illustrated in Fig. 2, following the repeated injections of the higher dose of THBC into the hippocampus, no significant differences were noted in the duration of the reaction between the first injection and the ones thereafter; however, the freezing-immobilization after the fourth injection of 51±29 sec was nevertheless significantly different from that of the CSF group, $t(14)=2.17$; $p<0.05$, in terms of the duration.

Motor Activity

As presented in Fig. 3, the micro-injection of the 10 ng dose of THBC (n=10) into the hippocampus reduced significantly the motor activity of the rats, $t(9)=3.2$; $p<0.05$. As measured by the number of squares crossed by the animals in the open field, the activity was about half of that observed following the control CSF-injection in the same animals. However, the 50 ng dose of the β-carboline did not cause a correspondingly significant decline in motor activity.

Grooming and Other Behavior

Table 2 shows that the hippocampal micro-injection of 10 ng and 50 ng doses of THBC enhanced substantially the interval of grooming during the 7.5 min test in the open-field test situation. The differences between the two dose groups and the CSF control groups were not statistically significant although the intervals of grooming were elevated following the β-carboline's injection into the hippocampus.

Other readily observed changes in behavior produced by the THBC micro-injections included vocalization to touch, crawling, biting, and tail rigidity. In addition, the periods of freezing-immobilization were typically characterized often by whole body tremor, lateral head-weaving, and a fixed gaze of the eyes. Finally, in 30% of the rats, we observed epiliptiform activity when the higher dose of the THBC was injected repeatedly.

DISCUSSION

The present study provides further evidence that the central effect of a β-carboline in inducing an anxiety-like behavior in the rat involves the limbic system [15,36]. Further, the results show that the hippocampus, which contains benzodiazepine receptors in high density [18], is highly reactive to the local presence of THBC. In relation to these findings is the study of Polc *et al.* [29] who demonstrated electrophysiologically that a β-carboline derivative applied to hippocampal neurons exerted an excitatory effect which was antagonized by a benzodiazepine applied to the same cells.

The anxiety-like state induced by the THBC microinjected into the hippocampus was characterized by a behavioral inhibition in an open field, resulting in freezing-immobilization and a reduction in motor activity. The lower dose of the THBC had a more pronounced effect on motor activity than that produced by the higher dose; however, the higher dose of THBC was more efficacious than the lower in terms of the intensity and duration of freezing-immobilization. Other results obtained with ICV injections correspond generally to the present results based on the direct application of the THBC to the hippocampus. For example, a low dose of THBC administered ICV can attenuate motor activity [15], whereas a higher dose of THBC may induce stereotyped behavior [1]. Interestingly, THBC in either dose did not alter the rate of defecation as measured by the number of excreted fecal boluses, which is considered to be a valid index of emotional reactivity of the animal placed in a novel environment [2, 9, 34].

Although the central mechanism of action of THBC is unknown, the decline in the rat's motor activity produced by a β-carboline may be due to a generalized anti-dopaminergic action of the harmane derivative [2]. Also, the effect of THBC could be related to an increase in the synaptic activity of monoamines within neurons of the rat's hippocampus [16] since THBC is known to inhibit the uptake of 5-HT and

catecholamines [17]. Alternatively, the dose-dependent difference in the behavioral changes following hippocampal THBC could result from the differential uptake of the β-carboline into one of the classes of benzodiazepine receptor. When a low concentration of [^{3}H]-propyl-β-carboline-3-carboxylate is injected into the rat's brain, a single benzodiazepine receptor binding site is labelled [13]. On the other hand, a higher concentration of this alkaloid derivative labels both high and low affinity benzodiazepine receptor sites [13]. The anxiolytic effect of a benzodiazepine may be mediated through the Type I benzodiazepine receptor and the sedative-hypnotic effect by any of the Type II class of receptor, but several reports in the literature are inconsistent with this viewpoint [14]. To illustrate, the hippocampus, which is considered to be a pivotal structure in the neurochemical mechanisms underlying emotional behavior [22], is populated predominantly by Type II benzodiazepine receptors [14] that are reportedly more reactive to the presence of GABA [13]. In addition, Braestrup and colleagues [7] have provided evidence that the Type II class of receptor may, in fact, subserve the central anxiety-like effects produced by an anxiolytic drug. On the other hand, the *in vitro* affinity of 1,2,3,4-tetrahydro-β-carboline to benzodiazepine receptors is very low [4,20], but nevertheless the compound exerts potent pharmacological effects centrally [4,24].

Certain β-carbolines exert a convulsive effect in rodents, suggesting that THBC can induce abnormal electrical activity in the brain [28,32]. From chemical mapping studies in several species, it is recognized that the hippocampus is a principal site of origin of epiliptogenic discharges [22]. In our experiments, an epiliptiform response was observed in some cases following the local micro-injection of THBC into the hippocampus. Since vocalization to touch and tail rigidity occur also in the rat following repeated micro-injections of THBC, the compound may bind to one of the opiate receptor types present in the hippocampus. When an opiate agonist is applied to this structure, this same pattern of responses can be elicited [8].

Finally, further research will be required to elucidate the pharmacological nature of action of a β-carboline on the hippocampus. For example, it will be essential to determine whether the blockade of benzodiazepine receptors as well as opiate receptors prior to the direct application of THBC and other β-carbolines will inhibit or attenuate their powerful actions.

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