Wild running and conditioned place aversion produced by microinjection of a GABA-A receptor antagonist into the inferior colliculus of the rat

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Blockade of GABAergic transmission within the inferior colliculus (IC) induces wild running (WR). WR was shown to present behavioral similarities with aversive reactions elicited from brain periventricular structures. The aim of the present study was to check whether blockade of IC GABAergic transmission could induce aversion. Microinjections of semicarbazide, a GABA synthesizing blocker, or SR95531, a GABA-A receptor antagonist, into the IC were performed. The elicitation of aversive effects was assessed in the place conditioning paradigm. Bilateral microinjection of semicarbazide (6 µg/0.2 µl) or of SR95531 (1 ng and 3 ng in 0.2 µl) elicited WR and tonic seizures. Carbachol (2 µg in 0.2 µl), a cholinergic agonist, elicited myoclonic seizures but not WR. The vehicle did not elicit any of these behavioral reactions. Microinjection of the same doses of semicarbazide and SR95531 into the IC elicited place aversion shown by a decrease in the time spent in the drug paired compartment during the postconditioning test. Microinjection of carbachol also elicited place aversion whereas saline or bicuculline did not. These results suggest that the blockade of the tonic inhibition exerted by GABAergic terminals within the IC results in WR, Tonic seizures and aversive effect.

Keywords: Inferior colliculus, GABA-A receptors, SR95531, Carbachol, Wild running, Epilepsy, Place conditioning, Aversion.

INTRODUCTION

Wild Running behavior (WR) constitutes an important component of audiogenic seizure (AGS). It is induced by a high intensity acoustic stimulation in AGS susceptible strains of rats and is often followed by a tonic (TS) [1] or a tonico-clonic seizure [2]. The initiation site in the neuronal network activated during WR is the inferior colliculus (IC). As a matter of fact, lesions of the IC are the most effective in suppressing WR [3] and electrical stimulation of the IC elicited WR behavior [4]. The epileptic nature of WR is manifested by a significantly increased spontaneous IC neuronal firing during the period of AGS susceptibility [5], a high incidence of sustained repetitive IC response patterns to the acoustic stimulus [6], and IC afterdischarge activity during WR occurrence [7]. Regarding neurochemical alterations, WR and AGS susceptibility may result from a decreased efficiency of IC GABAergic transmission. Local microinjection of muscimol [8], a GABA-A agonist, or tiagabine [9], which blocks the uptake of GABA, into the IC reduced WR and seizure severity. Blocking the GABAergic transmission by microinjections of GABA-A antagonists into the IC elicits WR [10]. From behavioral observations, it was suggested that WR presents many similarities with the flight reaction or the defense reaction elicited by the activation of the periaqueductal gray (PAG) and the superior colliculus (SC) [10, 11]. In contrast with WR, these behavioral
responses have not been associated to any epileptic activity. Stimulation of PAG or SC was shown to generate an aversive effect [12,13]. Therefore, the question of whether activating the IC enough to induce WR may generate an aversive effect has to be asked. Indeed, we found, in an operant conditioning situation, that IC electrical stimulation produces an aversive effect since rats learned to stop IC stimulation before WR could start [14]. In the present study we verified that IC activation, via a GABAergic transmission blockade, could also induce an aversive effect. Microinjections of semicarbazide, a GABA synthesizing blocker, and SR95531 (gabazine), a GABA-A receptor antagonist [15], into the IC were performed. The place conditioning paradigm, was used for assessing the aversive effects [16]. Place conditioning has already been successfully used to study both the aversive and the rewarding properties of intracranial microinjections of drugs [17, 18]. Furthermore, we verified the specificity of the excitatory effects of GABA-A antagonists by testing the effects of another excitatory agent [19], carbachol, a cholinergic agonist.

MATERIAL AND METHODS

Animals and surgery

Fifty-six naive male rats (Long Evans, Janvier, France) weighing 250 to 350g were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with the European Communities Council Directives (86/609/EEC). Each rat was implanted bilaterally with a stainless-steel guide canula (0.4 mm o.d., 0.3 mm i.d.) under ketamine anesthesia (125mg/kg i.p.). The canulae had coordinates (using lambda as the reference for each plane) of : -0.8 mm anterior, 1.8 mm lateral, 4.5 mm ventral. The canulae were anchored to the skull using polycrlic cement and three stainless-steel screws. They were plugged with stainless-steel stylets and protected with a tube (diameter: 10 mm, height: 5 mm) vertically maintained on the skull of the rat with cement. Each rat was housed in its individual cage with ad lib food and water supply in a colony room maintained on a 12/12 hr. light-dark cycle and was allowed to recover for one week following the implantation.

Apparatus

Each animal was tested and conditioned in one of four wooden place conditioning enclosures described in a previous study [18]. This apparatus consisted of three compartments. Two large (45x45x30 cm) compartments (compartments A and B) separated by a wooden wall had distinctive walls and floor and a plexiglas front through which the animals were observed from another room using a video camera. One compartment had black and white striped walls, a white ceiling and a plexiglas floor covered with wooden chips, the other one had black walls, a black ceiling and a wire grid floor. The third compartment C was adjacent to both A and B, and was painted gray including the ceiling, and had a removable wooden partition. When the partition was in place, the rat was confined to one of the large compartments. When the partition was removed the rat could freely move between the two large compartments via compartment C.

Procedure

The procedure was divided into three main phases and lasted 10 consecutive days referred at as « day 1 » to « day 10 » hereafter. Before conditioning (preconditioning test: day 1) and after conditioning (post conditioning test: day 10), rats in a drug-free state are placed in the apparatus and given free access to the compartments. During conditioning (day 2 to day 9), rats receive one distinct environment in compartment A or B paired with a drug and in a distinctly different environment in the other compartment, the rat receives sham injection. This protocol is repeated for 4 exposures to each environment. A drug or a stimulus is considered to have rewarding properties if the rat spends more time in the paired compartment during the post conditioning test. Conversely, a drug or stimulus has an aversive property if the rat spent less time in the paired compartment.

Behavioral testing and conditioning started after daily handling sessions. Each rat was injected and submitted to conditioning or testing during the same time of the day.
1. Preconditioning test
The partition being removed, the position of the rat within the apparatus was recorded using infrared photocells that provided continuous information on the position of the rat to a microcomputer programmed to measure and stored the time spent in each compartment.

2. Conditioning
On days 2, 4, 6 and 8 half of the animals were sham-injected; on days 3, 5, 7 and 9 they were microinjected with saline or a drug. The procedure was reversed for the other half of the rats. The rats were confined for a 30 min. period in compartment A or B, the number of animals experiencing saline or a drug in A being counterbalanced with the number of animals experiencing it in B. All drugs were dissolved in saline (0.9%), the pH being raised to 7 whenever necessary with a few drops of sodium hydroxide. Six groups of rats were submitted to that procedure. Each group of rats was injected respectively by: SAL, 0.2 µl saline 0.9 %; BM, bicuculline methiodide (Sigma) 10 ng / 0.2 µl; CA, carbachol (Sigma) 2 µg / 0.2 µl; SCBZ, semicarbazide (Sigma) 6 µg/ 0.2 µl; SR1 or SR3, : SR95531 [(2-(3'-carboxy-2'-propenyl)-3-amino-6-paramethoxy-phenyl-pyridazinium bromide), provided from the Laboratoire de Pharmacochimie, Centre de Neurochimie, Strasbourg], respectively 1 ng or 3 ng / 0.2 µl. For the microinjections, the rats were gently handled, the stylets were removed and an injection canula (0.28 mm o.d., 0.18 mm i.d.) was inserted into each guide canula. When in place, the injection canulae protruded 1 mm the guide canulae. A volume of 0.2 µl was bilaterally injected over 30 sec, then the stylets were put back in place. For sham-injections, care was taken to ensure a similar handling of the rats as for microinjections. The rats being gently hand-held, the stylets were removed and replaced over a period of time similar to that of a real microinjection. After SAL, BM, CA, SR1, SR3, or sham-injections, the rats were confined immediately in the appropriate compartment whereas after SCBZ microinjections the rats were confined 10 minutes later.

On days 2 and 3, behavioral effects of the microinjections were recorded on videotapes. Later on, the latency of onset and the duration of the following behavioral responses were measured: intense locomotor activity (i.e. walking phase of unusual long duration, lasting more than 4 minutes), wild running, tonic seizures (described in [20]) and myoclonic seizures.

3. Post-conditioning test
On day 10, the rats were placed in the shuttle compartment (C) with the partition removed and their position was recorded over a 15 min. period exactly as it was during the pre-conditioning test.

Histology
On completion of the experiments, the rats were sacrificed with an overdose of pentobarbital and perfused intracardially with saline followed by 4% formalin. Serial 20 µm brain sections were stained with cresyl violet and the locations of the injection sites were determined using the corresponding planes of the Paxinos and Watson Stereotaxic Atlas. Due either to loss of their guide canulae or clogging of one of them, the number of rats remained in each group at the end of the experiment was: SAL, N=10; BM, N= 8; CARB, N=10; SCBZ, N=7 ; SR1, N=11 and SR3, N=8. Results obtained with these animals were submitted for statistical analysis.

Statistics
The incidence of the behavioral effects of drug (or vehicle) microinjection was compared to that of a sham injection in the same rat by use of the Mc Nemar test [21]. The time spent in the drug paired compartment obtained during the post-conditioning session was compared to the same parameter obtained during the pre-conditioning by use of a paired T test (BMDP statistical software [22]).
RESULTS

Figure 1 shows that every site used that could be clearly identified through the histological procedure was located within the inferior colliculus or close to it.

Figure 1: Location of microinjections sites on planes of the Paxinos and Watson Atlas. The various groups are represented by the following symbols: □, SCBZ (semicarbazide); ▲, SR1 (SR95531 : 1 ng); ●, SR3 (SR95531 : 3 ng); ■, BM (bicuculline methiodide; △, CARB (carbachol). Symbols : IC = inferior colliculus, PAG = periaqueductal gray, SC = superior colliculus.

The behavioral effects elicited after the first microinjection during the initial conditioning sessions (day 2 or 3) are presented in Figure 2. Bilateral microinjection of SCBZ induced WR in all the rats of the SCBZ group and microinjection of SR95531 also induced WR in rats of SR groups. Furthermore, in a percentage of these rats WR progressed to TS (Figure 2). In contrast, microinjections of BM, SAL or CARB did not elicit WR. The incidence of WR after SCBZ was significant (X2 = 9.09, 1 df, p < 0.01). Following SR1, WR started as soon as the first minute after the end of the microinjection and lasted 30 to 35 minutes. The dose of 10 ng of BM elicited periods of freezing behavior and the duration of its effect was 10 minutes. In the CARB group, the microinjections elicited a qualitatively different behavioral response which consisted in an intense locomotor activity (in 95% of the rats tested, X2 = 7.11, p < 0.01) which turned in some instances (20% of the rats) to myoclonic seizures consisting of recurrent myoclonic convulsions of the face, the head and the whole body associated with rearing and falling.

Figure 2: Incidence of wild running behavior (white bars) and wild running that ended in tonic seizures (cross hatched bars) induced by bilateral microinjections of SCBZ, SR95531, SAL, BM and CARB into the IC.

During the pre-conditioning test, the rats of the six groups tested spent a similar amount of time in each of the large compartment (x ± s.e.m = 306.2 ± 10.0 sec and 311.6 ± 10.0 sec.). Microinjection of SCBZ into the IC elicited place aversion as shown by a decrease as the first minute after the end of the microinjection and lasted 30 to 35 minutes. The incidence of WR after SR3 was significant (X2 = 9.09, 1 df, p < 0.01). Following SR1, WR started as soon as the first minute after the end of the microinjection and lasted 30 to 35 minutes. The dose of 10 ng of BM elicited periods of freezing behavior and the duration of its effect was 10 minutes. In the CARB group, the microinjections elicited a qualitatively different behavioral response which consisted in an intense locomotor activity (in 95% of the rats tested, X2 = 7.11, p < 0.01) which turned in some instances (20% of the rats) to myoclonic seizures consisting of recurrent myoclonic convulsions of the face, the head and the whole body associated with rearing and falling.
in the time spent in the drug paired compartment during the post-conditioning test \( (t = 4.18, p < 0.05) \) (Figure 3). Microinjection of SR95531 into the IC elicited place aversion as shown by the significant decrease in the time spent in the drug paired compartment during the post-conditioning test \( (t = 3.14, p < 0.05 \text{ for SR1 and } t = 3.28, p < 0.05 \text{ for SR3}) \) (Figure 3). It appears from Figure 3 that microinjection of carbachol elicits a significant decrease in the time spent in the drug paired compartment \( (t = 2.7, p < 0.05) \). The effects of the microinjection of BM or of saline did not reach a level of significance.

**DISCUSSION**

The present study shows that microinjection of SCBZ or of SR 95531 into the IC induces WR and TS as well as aversive effects. These effects may result from a focal action within the IC. SCBZ and SR95531 induced-WR extend results of previous studies showing that IC microinjections of GABA antagonists induced WR. These effects could not result from a diffusion of the drug to the nearby periaqueductal gray (PAG) or superior colliculus where GABA-A antagonists induced similar behavioral effects [23]. The effective sites were located far from these structures and SR95531 induced-WR has a rapid onset, before any diffusion of the very small volume microinjected could occur [8]. Moreover, GABA-A antagonists were shown to directly excite the IC [24, 25] and to induce WR although removal of the whole PAG [10].

SCBZ inhibits the activity of the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD) [26] and it is likely that both WR and place aversion elicited by SCBZ may result mainly from a blockade of GABAergic transmission since muscimol abolish these effects [18]. SR95531 has a specific and potent GABA-A receptor antagonist action as shown by pharmacological, biochemical and electrophysiological studies [27, 28, 29]. Our results are consistent with a specific action on IC GABAergic synapses as WR and TS induced by SCBZ or SR95531 were qualitatively similar to those induced by bicuculline when injected into the same area within the IC [24]. Furthermore, SCBZ and SR95531 were microinjected within the central nucleus and the dorsal and lateral cortex of the IC known to contain GABAergic interneurones [30, 31] and GABA-A receptors [32]. Concerning the biochemical specificity, it is noteworthy that carbachol microinjections elicited different type of seizure [33] and produced a conditioned place aversion. These effects are mediated by an activation of the IC cholinergic transmission [19]. Furthermore, as Carbachol has an excitatory effect on IC neurons [19] our results suggests that activation of the IC resulting either from a release of an inhibitory effect or from an excitatory effect within the IC may generate an aversive effect.

Conditioning of drug effects in a place conditioning paradigm depend on the number of pairings, on the duration of each pairing, and on the temporal pairing of the effect of the drug with exposure to a particular environment [16]. The use of four pairings of 30 minutes duration in the present study was consistent with those most commonly used [16].
Microinjection of SCBZ or of SR95531 into the IC elicited conditioned place aversion whereas the dose of BM used did not. The doses used for SCBZ and of SR95531 induced behavioral effects lasting for all the exposure time in the confining compartment. This result suggests that aversion was induced which supporting an associative learning. Blocking specifically GABAAergic transmission within the IC elicited an aversive effect is consistent with electrical stimulation sustaining switch-off learning [4] and occurrence of autonomic responses, typical of the defense reaction, concomitantly with WR [11]. From these observations, it could be suggested that the IC shares the property of generating aversive effect with the mesencephalic structures of the brain aversive system, the PAG and deep layer of SC.

BM at the dose of 10 ng when injected into the IC elicits neuronal hyper excitability [24] and freezing behavior. The lack of a significant aversive effect elicited by BM may result from incomplete temporal pairing of the effect of the drug with the drug compartment. In fact, the 10 min duration of the effect of BM was not sufficient for a complete pairing with the 30 min exposure to the drug compartment. Unfortunately, we tested a higher dose of BM (35 ng) which induced WR for 30 min, but the explosive reactions elicited make difficult to complete the conditioning.

Strong acoustic stimulation induces aversion as animals learn to switch-off loud noise [34]. Of necessity, some parts of the auditory brain must contain, or be connected with, mechanisms relating to the genesis of aversion. The contrast between the strong affective responses produced by collicular stimulation [35], shown to sustain switch-off behavior [4, 14], and the lack of affective responses from even stronger geniculate stimulation implies a divergence of the pathways mediating aversive reactions from pathways mediating other auditory functions at the level of the IC or just above [35]. By microinjecting GABAergic drugs into the IC we got evidence that GABA-A receptors are involved in the control of auditory inputs to the IC [24]. Furthermore, microinjecting GABA-A receptor antagonists into the IC elicits WR that might be the overt expression of an aversive effect [10]. The striking correlation between the incidence of WR and the strength of place aversion reported in the present study (see Figure 2 and Figure 3) suggest that aversive effects are generated while WR occurs. Thus aversion, WR and TS could result from activation of the same IC neuronal network which is under the control of a tonic GABAergic inhibition.

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REFERENCES

10. Bagri A, Di Scala G, Sandner G. Wild running elicited by microinjections of bicuculline or morphine into the inferior colliculus of rats: lack of effects of


