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Escape Behavior Produced by the Blockade of Glutamic Acid Decarboxylase (GAD) in Mesencephalic Central Gray or Medial Hypothalamus

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BRANDAO, M L , G DI SCALA, M J BOUCHET AND P SCHMITT *Escape behavior produced by the blockade of glutamic acid decarboxylase (GAD) in mesencephalic central gray or medial hypothalamus* PHARMACOL BIOCHEM BEHAV **24**(3) 497–501, 1986 —Microinjections into the mesencephalic central gray (CG) or the medial hypothalamus (MH) of three drugs (L-allylglycine, Semicarbazide or 4,5 dihydroxy-isophtalic acid) known to block glutamic acid decarboxylase (GAD) produced a dose-dependent behavioral activation accompanied by jumps These effects are qualitatively similar to those produced by microinjections of SR 95103 (a GABA-A receptor antagonist) at the same sites These findings suggest that, at both the level of the CG and the MH, γ-aminobutyric acid (GABA) tonically inhibits a neuronal substrate involved in the generation of flight reactions

Periaqueductal gray Medial hypothalamus Microinjections Flight Escape GABA GAD inhibitors Rat

PREVIOUS studies [6,20] have shown that GABA receptor antagonists such as bicuculline methiodide [5] or SR 95103 [3] produce a behavioral activation together with jumps when injected in the medial hypothalamus (MH) or the dorsal part of the central gray (CG) However, the precise nature of such behavioral reactions thus induced differ, depending on whether the injection is placed in the MH or in the CG Microinjections in the MH were found to produce an increase of locomotor activity and of rearings, and such animals exhibited well-coordinated jumps also When placed in the CG, similar microinjections produced an increase of locomotor activity, but no increase in the number of rearings, and jumps were poorly coordinated and more explosive In addition, these rats showed an asymmetry in their reactions to tactile stimuli These data led to the suggestion that, at the level of both neural structures, GABA tonically inhibits the neuronal substrate involved in the generation of flight reactions and perhaps in the generation of an underlying aversive state However, bicuculline seems to act on both synaptic and non-synaptic sites [10] as, for example, bicucculline-sensitive sites have to be found to be located along axonal trunks [2] Thus, demonstrations that microinjections of bicuculline produce flight reactions do not necessarily mean that these behavioral reactions result from a blockade of the release of GABA

The aim of the present study was to complement the findings with GABA receptor antagonists by examining whether microinjections into the CG or the MH of drugs known to block the synthesis of GABA were efficient in eliciting behavioral reactions similar to those found after microinjections of GABA receptor blockers We studied the effects of three drugs known to block glutamic acid decarboxylase (GAD), the enzyme responsible for the GABA synthesis The three drugs employed, namely L-allylglycine [1, 13, 18], semicarbazide [15] and 4,5-dihydroxy-isophtalic acid [8] differed either in terms of their potencies and/or in the mechanism by which they produced a blockade of GAD

METHOD

Animals and Surgery

The experiments were performed on male Wistar rats (300–400 g) kept on a light dark cycle (12 12) and housed in individual cages with ad lib food and water

Each animal was anesthetized with pentobarbital (40 mg/kg IP) and fixed into a stereotaxic apparatus One stain-

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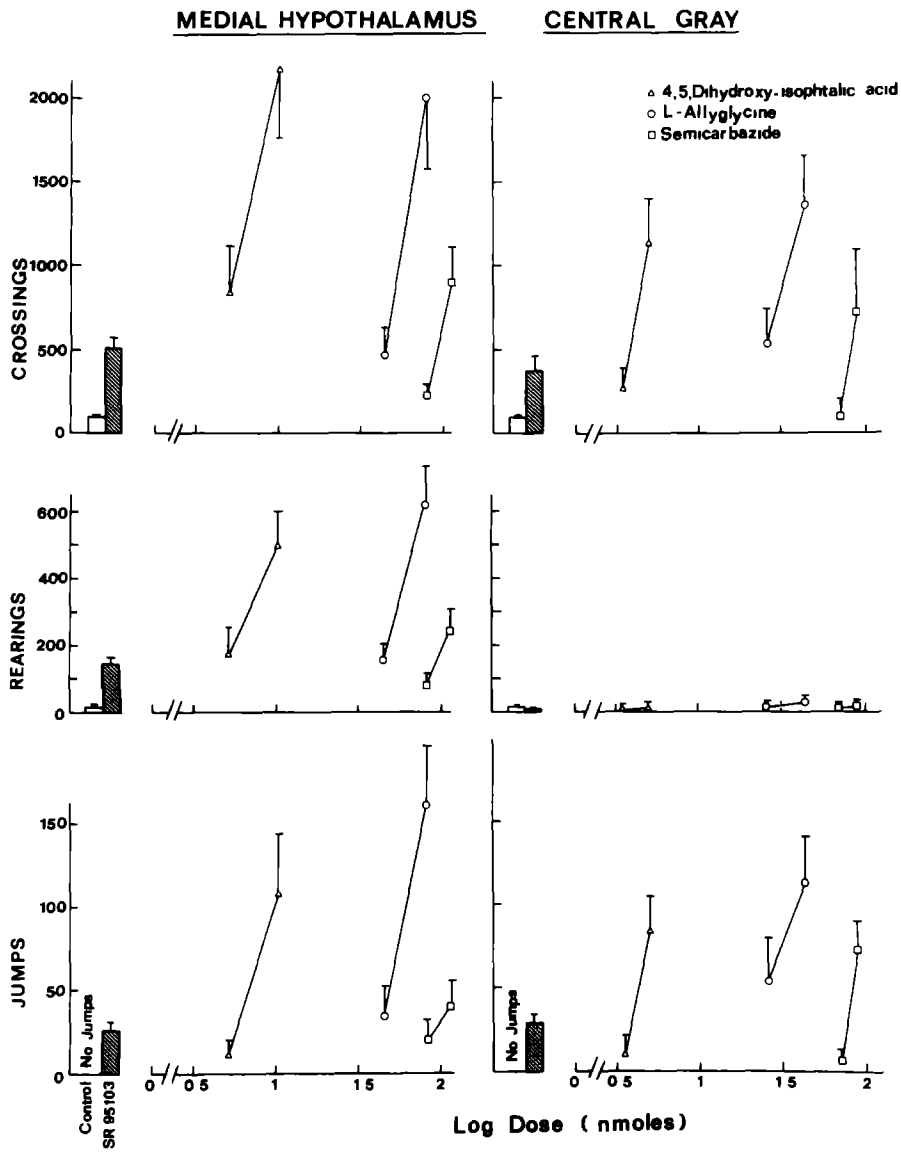

FIG 1 Mean total incidence (±SEM) of each of the three recorded behavioral responses following microinjection of phosphate buffer (control), 80 ng of SR 95103 or two different doses of either 4,5 dihydroxy-isophtalic acid, L-allylglycine or semicarbazide into the medial hypothalamus (left) or the central gray (right)

less steel guide-cannula (0 4 mm o d , 0 3 mm i d) was implanted into either the MH or the CG at the following coordinates, using the lambda point as the reference for each plane

	PA	ML	DV
MH	4 5 mm	0 3 to 0 5	7 5
CG	0 4 to 0 8	1 2	4 5 (with a medio lateral angle of 10°)

The guide-cannula was anchored to the skull by means of an autopolymerizing resin and three stainless-steel screws At the end of the surgery, the guide-cannula was sealed with a stainless steel wire

Drugs

SR 95103 was used at the dose of 80 ng (0 26 nmol) [20] Two behaviorally active doses for each of the three GAD inhibitors used were determined in pilot experiments L-allylglycine (Sigma) was injected at the dose of 5 μg (43 5 nmol) or 9 μg (78 nmol) at MH sites, and 3 μg (26 nmol) or 5 μg at CG sites, semicarbazide (Sigma) was injected at the dose of 9 μg (80 nmol) or 12 μg (110 nmol) at MH sites, and 8 μg (71 nmol) or 10 μg (89 nmol) at CG sites 4,5-dihydroxy-isophtalic acid was injected at the dose of 1 μg (5 nmol) or 2 μg (10 nmol) at MH sites, and 0 7 μg (3 5 nmol) or 1 μg at CG sites L-allylglycine (ALLY) was dissolved in sterile 0 02 M Phosphate buffer, Semicarbazide (SCB) and 4,5-dihydroxy-isophtalic acid (DHIP) were dissolved in

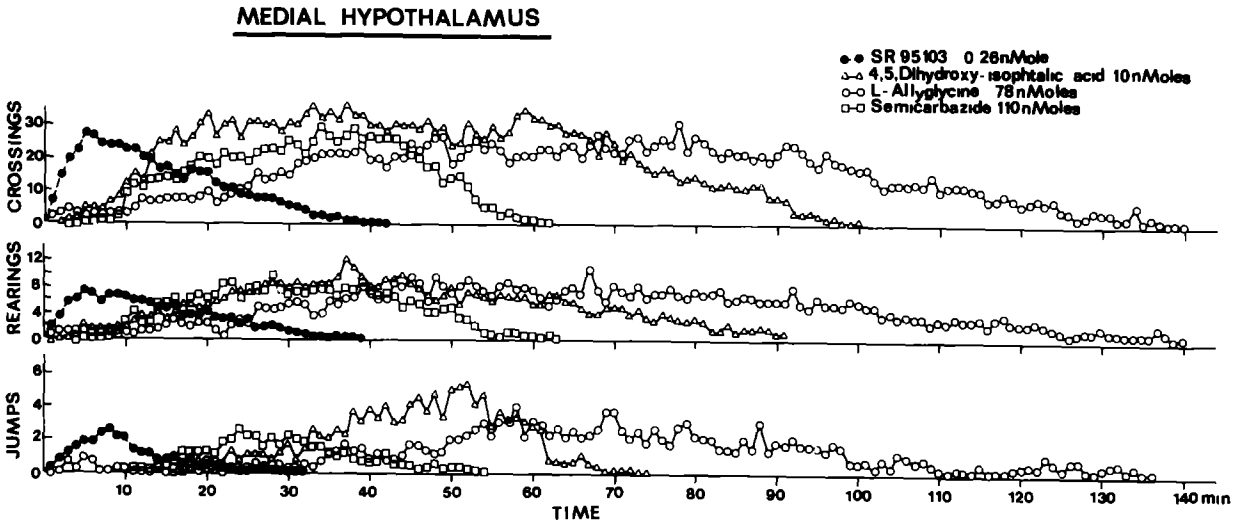

FIG 2 Time course of the behavioral effects (counts per minute) produced by microinjections into the medial hypothalamus (MH) of either SR 95103, or the higher dose of either 4,5 dihydroxy-isophtalic acid, L-allylglycine or semicarbazide

sterile distilled water The pH was adjusted to 7 0 by adding NaOH All the drugs were injected in a volume of 0 2 μl at a rate of 0 2 μl/20 seconds

Experimental Procedure

Following a postoperative delay of 1 week, each rat was placed for at least two hours in a circular enclosure (60 cm in diameter and 30 cm high with a floor divided into 12 sections) and allowed to explore the area The next day, each animal was returned to the enclosure Half an hour later, the steel wire was replaced by a stainless steel injection-cannula (0 28 mm o d , 0 18 mm i d) which protruded 1 0 mm beyond the tip of the guide-cannula and which was linked to a 1 μl Hamilton syringe by means of polyethylene tubing Each rat was then returned to the middle of the area and injected with SR 95103 The injection cannula was gently removed 1 minute following the end of the injection. The following behavioral responses were recorded every minute number of crossings (i e , number of floor sections traversed), number of rearings either against the wall or in the middle of the cage, number of jumps and, in some cases, rotations The rat was considered to jump either when it performed vertical jumps reaching the height of the enclosure's wall or when it actually jumped onto the top of the enclosure's wall In the latter case, it was gently taken back to the center of the enclosure Only those rats in which injection of SR 95103 produced at least one jump were subsequently injected with drugs to block GAD

These rats were assigned to three groups depending on the compound injected Half of the rats in each group received a MH injection, the other half a CG injection The two doses of a given drug were injected randomly at a given site with a delay of at least two days separating the two injections Ten MH and ten CG sites were submitted to a control-injection of 0 2 μl Phosphate buffer

For statistical comparisons, analyses of variance were used after a transformation to equalize the variances [7,23] These analyses were followed when appropriate by Bonferroni tests [7] In some instances, non-parametric tests were used [21]

Histology

On completion of the experiments, the animals were killed with an overdose of pentobarbital and intracardially perfused with saline followed by 10% formalin Serial 20 μm brain sections were stained with cresyl violet in order to localize the injection sites

RESULTS

At forty-eight sites, 24 for each structure, the microinjection of 0 26 nMole of the GABA-A receptor blocker SR 95103 produced a behavioral activation together with jumps (Fig 1) in agreement with previous findings [20] As described previously [6,20], these sites were found to be located either in the dorsal and dorsolateral part of the CG or in the dorsomedial and posterior hypothalamus The data obtained for each of the three recorded behavioral responses (crossings, rearings, jumps) were subjected to a two way analysis of variance (structure × group) No significant differences could be detected between groups There was a significant structure effect but only for rearings, $F(1,42=45\ 92, p<0\ 001$, the number of rearings being higher after MH than after CG injections Although there was no difference in the number of jumps, they were well-coordinated after MH injections whereas jumps were poorly coordinated and more explosive after CG injections

When injected either into the MH or into the CG, ALLY, SCB and DHIP each produced a behavioral activation together with jumps For the two structures studied, an analysis of variance was performed on the data obtained following control injections and injections of the higher dose of each drug tested For the number of crossings, a significant drug effect was found both at MH, $F(3,33)=49\ 4$, $p<0\ 001$, and at CG sites, $F(3,33)=40\ 4$, $p<0\ 001$ For the number of rearings, there was a significant drug effect at MH sites, $F(3,33)=32\ 5$, $p<0\ 001$, but no drug effect at CG sites,

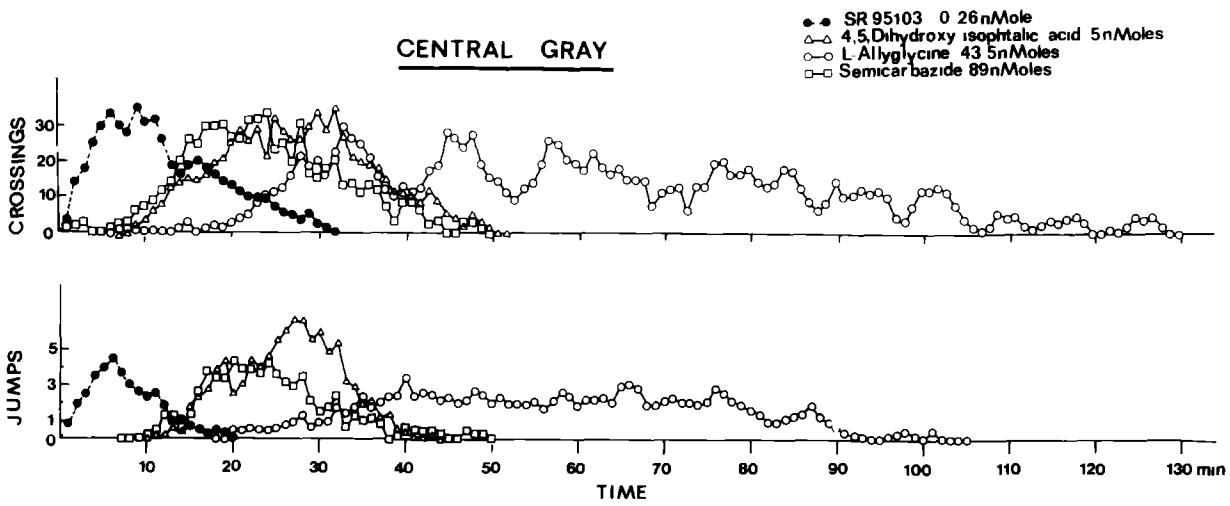

FIG 3 Time course of the behavioral effects (counts per minute) produced by microinjections into the central gray (CG) of either SR 95103, or the higher dose of either 4,5 dihydroxy-isophtalic acid, L-allylglycine or semicarbazide (Because of their small number, the rearings were not shown)

$F(3,33)=2\ 54, p>0\ 05$ Furthermore, for all drugs, the number of crossings, of rearings or of jumps were significantly higher following injection of the high dose (Wilcoxon $T=0, p=0\ 01$ in each case) than the corresponding number of behavioral reactions induced by the low doses, except for the rearings induced by CG injections

To estimate the potency of each drug, an arbitrary value was chosen for each behavioral measure and the dose that had to be applied in order to reach this arbitrarily fixed value was determined graphically using the data reported in Fig 1 For example, to obtain a total number of crossings which amounted to 900, either 5 3 nMoles of DHIP, 54 nMoles of ALLY or 110 nMoles of SCB had to be injected at MH sites Using such criteria, it was found that DHIP was 5 to 10 times more potent than ALLY, and 17 to 21 times more potent than SCB, depending on the behavioral measure and the structure considered

The time course of the effects produced by a microinjection of either SR 95103 or of each GAD inhibitor at the higher dose is shown on Fig 2 for the MH sites, and on Fig 3 for the CG sites There was a clear difference in both the delay of action of each drug (for crossings, $F(3,47)=85\ 0$ and 47 4 for MH and CG sites, respectively, $p<0\ 001$ in both cases) and the duration of the induced effects (for crossings, $F(3,47)=62\ 8$ and 53 5 for MH and CG sites, respectively, $p<0\ 001$ in both cases) Both the delay of action and the duration of the effects of a SR 95103 injection were significantly shorter than those of each of the three other drugs (Bonferroni $p<0\ 05$) Both the delay of action and the duration of the effects of ALLY injection were significantly longer than those of each of the three other drugs It can be added that there was also a difference, but a small one, between the effects of the drugs if one considers the mean number of crossings per minute ($F(3,47)=3\ 13$ and 3 96 for MH and CG sites respectively, $p<0\ 05$ in either case) The mean number of crossings per minute induced by DHIP injections was significantly higher than that induced by SR 95103 at MH sites, and significantly higher than those induced by either SR 95103 or ALLY at CG sites (Bonferroni $p<0\ 05$) When injected at CG sites each drug produced rotations contralateral to the stimulation site (data not shown) These rotations mainly appeared before and at the end of the locomotor activation In addition, the rats showed a neglect to tactile stimuli applied ipsilateral to the injection site whereas they showed a hyperreactivity characterized by withdrawal and jumps when the stimulus was applied contralateral to the injection site Also, the jumps were different when the injection was made in the MH or in the CG In the case of MH injections, the jumps were well oriented towards the top of the enclosure's wall whereas they were not so oriented and rather explosive following CG injections

DISCUSSION

The present data show that microinjections into periventricular structures of either L-allylglycine, semicarbazide or 4,5-dihydroxy-isophtalic acid produce a dose dependent behavioral activation accompanied by jumps These effects were qualitatively similar to those which follow a microinjection of SR 95103, a GABA-A receptor antagonist, at the same sites [3] Of particular interest is the fact that the clear difference which was found whether GABA-A receptor antagonists was injected into the MH or into the CG [6,20] was also found when ALLY, SCB or DHIP was injected either into the MH or into the CG

The most important differences between the effects of either bicuculline or SR 95103 injections on the one hand, and ALLY, SCB or DHIP injections on the other hand were in both the delay of action and the duration of effects Thus, some of the effects (e g , the increase of locomotor activity) appeared almost immediately at the end of the injection of SR 95103 or bicuculline whereas the delay was much longer for ALLY, SCB and DHIP The latter drugs have all been described to block, although through different mechanisms, the activity of GAD, the enzyme responsible for the synthesis of GABA [1, 8, 9, 15, 18] It is thus quite reasonable to assume that the behavioral activation and the jumps resulted from a local blockade of GABA synthesis at the level of the CG or the MH Two sets of data are of particular interest in this context

(1) ALLY was found to have both the longest delay of action and the longest duration of effects Numerous studies

have already noted that the inhibition of GAD by ALLY appears after an especially long delay, both in vivo [11, 17, 22] and in vitro [9] Also the brain level of GABA is correspondingly decreased for a long time following ALLY [17,19] To explain these findings, it has been suggested that ALLY does not act on GAD by itself but through a metabolite, namely 2-keto-4-pentenoic acid [18] which has indeed been found to inhibit GAD [12,19] and to be more potent than either L- or D-ALLY [12,14]

(2) The three drugs used in the present work showed a different efficiency in eliciting the behavioral activation following microinjections Thus, when the total increase in the recorded behavioral parameters is considered, it appears that DHIP is 5 to 10 times more potent than ALLY and 17 to 21 times more potent than SCB Interestingly, when one considers the potency of each of these compound to inhibit GAD in vitro, a similar ranking appears Thus, DHIP (K_i=0 18×10^{-6} M) [8] is the most potent one, whereas SCB (K_i=130±28$\times10^{-6}$) is the less potent, the potency of 2-keto-4-pentenoic acid (K_i=2 4×10^{-6} M) [19], the believed active metabolite of ALLY being in between

Taken together these considerations strongly support the idea that, at the level of both the MH and the CG, a GABAergic synaptic mechanism tonically inhibits a neural substrate involved in the generation of flight behavior and perhaps in the elaboration of an underlying aversive effect It should be added that behavioral reactions which present some similarities with those obtained following CG microinjections, but not with those obtained following MH microinjections, have been described following microinjections into the superior colliculus of either tetanus toxin [16] which reduces the release of GABA [4], or semicarbazide [24] This suggests a functional similarity for both the CG and the superior colliculus, at least for some of their functions

It is well known that systemic injections of GAD inhibitors induce convulsions often preceded by running episodes The present data and those described above [16,24] suggest that these running episodes might be due to an action of these GAD blockers at the CG or superior colliculus level rather than at the MH level

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