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An *In Vivo* Method for Testing GABAergic Compounds

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FUNG, Y. K., A. NOVIN-BAHERAN, T. K. BOSH AND D. R. GRASSMAN. *An* in vivo *method for testing GABAergic compounds*. PHARMAC. BIOCHEM. BEHAV. 17(4) 651–654, 1982.—This report describes an intracerebroventricular technique of drug injection which enables compounds with GABAergic properties to be rapidly identified *in vivo*. In addition, this method allows for the testing of compounds that poorly penetrate the blood brain barrier for GABAergic activities. Subcutaneous administration of apomorphine (1.5 mg/kg, SC) elicits climbing behavior in mice. The apomorphine-induced climbing behavior is inhibited by the intracerebroventricular or intraperitoneal administration of known GABA (gamma aminobutyric acid) agonists including muscimol (10–50 ng) and GABA (2–10 μg). This inhibitory effect of GABA or muscimol on apomorphine-induced climbing behavior can be reversed by picrotoxin (2 mg/kg, IP), a known GABA-receptor antagonist.

GABA Climbing behavior *In vivo* studies Muscimol Apomorphine Picrotoxin

MICE treated with low doses of apomorphine exhibit climbing behavior when placed in wire mesh metal cages. This peculiar behavior appears to be elicited by direct stimulation of dopamine receptors in the striatal and mesolimbic regions [2,13]. Both the nigrostriatal and mesolimbic dopaminergic neurons have been implicated in the regulation of locomotor function and their activities are believed to be regulated by GABAergic neurons [6,11]. Since local application of GABA on nigral dopaminergic neurons of rats has been shown to inhibit the rate of firing of dopaminergic cells [1,12], this suggests an inhibitory role of GABA on the dopaminergic system.

This paper describes a rapid method of potential value for screening and testing potential GABAergic compounds (GABA agonists that directly activate the GABA receptors or compounds that increase the release of GABA from nerve terminals). The influence of GABA on central dopaminergic system was studied by the direct central or systemic administration of GABAergic agents and examined for their effect on apomorphine-induced climbing behavior in mice.

METHOD

Animals

Male ICR mice (Harlan, IN) weighing between 23–30 g were used. They were allowed free access to food (Purina Lab. Chow) and water. All mice were housed in plastic cages (10/cage) in a room maintained at 23±1°C with an automatic 12-hr. light-dark cycle.

Intraperitoneal (IP) Drug Administration

Animals were pretreated with either saline, GABA (100–600 mg/kg, IP) or muscimol (0.25–0.8 mg/kg, IP) 5 minutes prior to the injection of apomorphine (1.5 mg/kg, SC).

Intracerebroventricular (ICV) Drug Injections

Mice were anesthetized with chloral hydrate (430 mg/kg, IP) and an incision was made in a longitudinal direction. A hole was made in the left side of the skull (1.2 mm lateral to bregma) for the ICV administration of various compounds at a later time.

Under halothane anesthesia, GABA (2–10 μg) or muscimol (10–50 ng) in a volume of 5 μl was injected into the left ventricle of the mouse via a 10 μl Hamilton syringe. The syringe was fitted with a polyethylene cuff so that only the distal 2.5 mm of the needle was exposed. This "free hand" injection was performed over a period of 20 seconds and the needle was held in place for an additional 10–15 seconds before withdrawing from the skull. The incision was then closed with a wound clip. All animals recovered from the halothane anesthesia within 2–3 minutes after the ICV injection. The location of drug in the brain was confirmed by examining the stain produced by the injection of a 10% methylene blue aqueous solution intraventricularly in a group of six animals.

Behavioral Assessment

Apomorphine-induced climbing behavior was employed to assess dopaminergic function [2,3,13]. Animals were observed in wire mesh metal cages that were 15 cm high, 12 cm in diameter and covered at the bottom with a metal plate. All mice were allowed to adapt to these cages for 30 minutes prior to the experiment.

Apomorphine (1.5 mg/kg, SC) was given five minutes after the ICV or IP administration of different drugs and the mice were observed for climbing behavior for the subsequent 30 minutes and were recorded for climbing index [3]. The "climbing index" (C.I.) is the percent of time spent in climb-

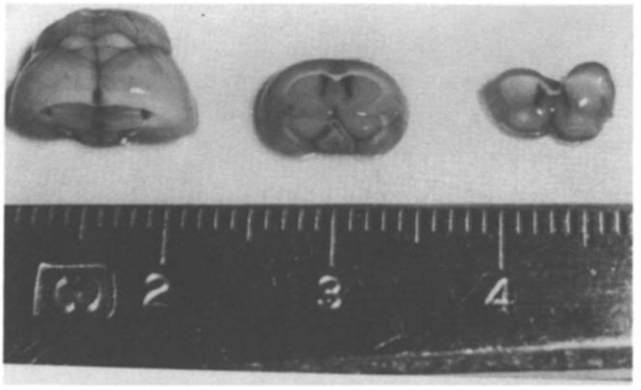

FIG. 1. Under halothane anesthesia, animals received intracerebroventricular injection of a 10% methylene blue solution (5 μl). Five minutes after injection, the mice were killed and sections were made near the site of injection. The stain followed the needle tract, but was mainly located at the ventricles.

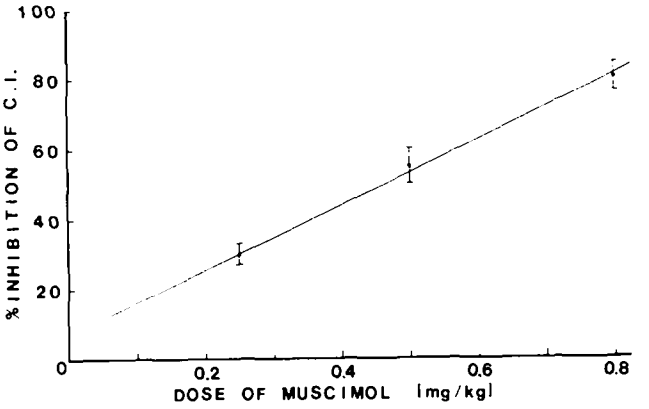

FIG. 2. Effect of intraperitoneal administration of muscimol (0.25–0.8 mg/kg, IP) on apomorphine-induced climbing behavior. Muscimol was given 5 minutes prior to the administration of apomorphine (15 mg/kg, SC) and the climbing index was measured after apomorphine injection. Each point represents the mean+S.E.M. of 6–7 observations as compared to controls which showed a C.I. of 82±5% (n=7).

ing during the 30 minute period after the first climb.

In some experiments, picrotoxin (2 mg/kg, IP) was administered 15 minutes prior to the ICV or IP administration of muscimol or GABA to examine its possible antagonistic effect on the inhibitory actions of muscimol or GABA on climbing behavior. All behavioral observations were made between 9:00 a.m. and 5:00 p.m.

Muscimol, GABA and picrotoxin were purchased from Sigma Chemical Company and were dissolved in saline solution. Apomorphine KCl (Merck Company, Inc.) was dissolved in 0.1% sodium metabisulfate. For ICV injection, the drug was given in a volume of 5 μl, while for intraperitoneal or subcutaneous drug injection, the compound was given in 0.1 ml/10 g animal body weight.

RESULTS

Site of Drug Injection

The ICV injection of a 10% methylene blue solution (5 μl) in mice confirmed the site of drug injection to be in the lateral ventricles (Fig. 1).

Effect of Intraperitoneal Administration of Muscimol or GABA

Intraperitoneal administration of muscimol (0.3–0.8 mg/kg) inhibited apomorphine-inducing climbing behavior in a dose-dependent manner (Fig. 2). The IC_{50} 50% inhibition of conversion index and the 95% confidence limit was determined to be 0.47 mg/kg (0.21–1.03). On the contrary, systemic injection of GABA up to 600 mg/kg was ineffective in inhibiting the apomorphine-induced climbing.

Effect of ICV Administration of Muscimol or GABA

All mice appeared normal after the ICV administration of either muscimol (10–50 ng) or GABA (1–10 μg). However, these treatments inhibited the apomorphine-induced climbing behavior (Figs. 3 and 4). The IC_{50} and the 95% confidence limits were determined graphically from the dose-inhibition curve and were as follows: muscimol 27 ng (16–47 ng), GABA 4.5μg (2.3–8.8 μg) [10].

Effect of Picrotoxin on the Inhibition of Climbing Behavior Produced by Muscimol or GABA

Picrotoxin (2 mg/kg, IP) administered 15 minutes before the ICV or IP injection of muscimol or GABA was found to be effective in antagonizing the inhibitory effect of these compounds on apomorphine-induced climbing. This is indicated by the reversal of the muscimol or GABA-induced inhibition of climbing index (Tables 1 and 2). Picrotoxin alone (2 mg/kg, IP) did not elicit convulsion, nor did it alter the climbing behavior produced by apomorphine.

DISCUSSION

Our studies show that ICV administration of either muscimol or GABA effectively inhibits apomorphine-induced climbing behavior. Intraperitoneal injection of muscimol but not GABA also inhibit apomorphine-induced climbing; this may be due to the poor penetration of GABA (an amino acid) across the blood brain barrier. Our findings are in accordance with Dunn *et al.* [4], who found an inhibitory effect of muscimol (IP) on apomorphine-induced climbing behavior. Muscimol is more potent than GABA in inhibiting the apomorphine-induced climbing behavior. This may be due to the poor penetration of GABA from the ventricular space into the brain tissue or high uptake of GABA into glial cells and GABA neurons [4,5]. In addition, muscimol has a greater affinity than GABA in binding to the GABA receptors [9].

Since the inhibitory effect of GABA and muscimol on apomorphine-induced climbing behavior can be reversed by the pretreatment of picrotoxin, a GABA antagonist, this suggests that the stimulation of GABA receptors is involved in the behavioral changes mediated by muscimol and GABA. Picrotoxin may act on the membrane molecule responsible for controlling the GABA-induced chloride flux, the GABA ionophones, or the link between receptors and ionophone, thus preventing the inhibitory effect of GABA on nerve tissues [9]. The exact mechanism whereby GABA-mimetics may act to suppress apomorphine-induced climbing behavior

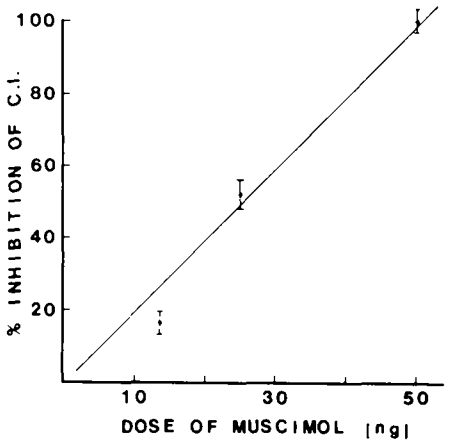

FIG. 3. Effect of intracerebroventricular injection of muscimol on apomorphine-induced climbing behavior. Under halothane anesthesia, muscimol (10–50 ng) in a volume of 5 μl was injected into the left ventricle of the mouse. Five minutes later, apomorphine (1.5 mg/kg SC) was given and the climbing index was measured. Each point represents the mean ± S.E.M. of 5 observations, as compared to saline controls which showed a C.I. of 86 ± 4% (n=5).

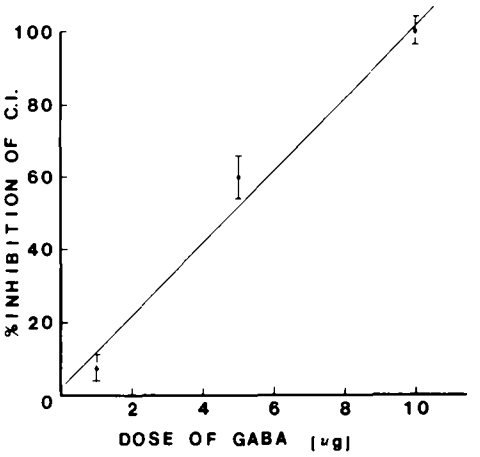

FIG. 4. Effect of intracerebroventricular injection of GABA on apomorphine-induced climbing behavior. Under halothane anesthesia, GABA (1–10 μg) was injected in a volume of 5μl into the left ventricle of the mouse. Five minutes later, apomorphine (1.5 mg/kg, SC) was given and the climbing index was measured. Each point represents the mean ± S.E.M. of 5 observations, as compared to the saline-treated controls which showed a C.I. of 83 ± 3% (n = 5).

TABLE 1

ANTAGONISM OF THE EFFECT OF INTRACEREBROVENTRICULAR ADMINISTRATION OF MUSCIMOL OR GABA ON APOMORPHINE-INDUCED CLIMBING BY PICROTOXIN

Treatment Groups	Climbing Index (C.I.)
Saline (IP) + Saline (ICV) +apomorphine (1.5 mg/kg, SC)	84 ± 4
Muscimol (50 ng, ICV) +apomorphine (1.5 mg/kg), SC)	8 ± 4*
Picrotoxin (2 mg/kg, IP) +muscimol (50 ng, left ventricle) + apomorphine	76 ± 8
GABA (10 μg, ICV) +apomorphine (1.5 mg/kg, SC)	7 ± 5*
Picrotoxin (2 mg/kg, IP) + GABA (10 μg, ICV) +apomorphine (1.5 mg/kg, SC)	69 ± 8

Mice were pretreated with either saline (IP) or picrotoxin (2 mg/kg, IP) 15 minutes before the ICV injection of muscimol or GABA. Five minutes after the ICV injection, all mice received apomorphine (1.5 mg/kg, SC) and the climbing index was determined as described in method section.

Results are expressed as mean + S.E.M. (N=5–6 in each group). *$p<0.05$ (Mann-Whitney "U" test) when compared to saline controls.

TABLE 2

ANTAGONISM OF THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF MUSCIMOL ON APOMORPHINE-INDUCED CLIMBING BY PICROTOXIN

Treatment Groups	Climbing Index (C.I.)
Saline (IP) + Saline (IP) apomorphine (1.5 mg/kg, SC)	83 ± 4
Muscimol (0.8 mg/kg, IP) −apomorphine (1.5 mg/kg, SC)	20 + 5*
Picrotoxin (2 mg/kg, IP) +Saline (IP) −apomorphine (1.5 mg/kg, SC)	76 ± 4

Mice were pretreated with either saline or picrotoxin 15 minutes before the administration of muscimol (0.8 mg/kg, IP) or saline. Five minutes later apomorphine (1.5 mg/kg, SC) was given and the climbing index was measured as described in method section.

Results are expressed as mean ± S.E.M. (N=5–6 in each group). *$p<0.05$ (Mann-Whitney "U" test) when compared to saline controls.

remains unclear. One possible explanation is that GABA-mimetic may act simply by inhibiting dopaminergic neurons in the nigrostriatal and mesolimbic regions [4], since stimulation of receptors in these areas has been implicated in the initiation of climbing behavior [2,13].

Our present procedure does not distinguish between a GABA agonist that directly activates GABA receptors from a compound that may increase the release of GABA from nerve terminals. Other methods available for testing putative GABAergic drugs include iontophoresis onto single neurons, receptor binding, and direct administration of GABAergic agents via a cannula into the globus pallidus of rats [7,14]. These methods have certain limitations. Implantation of cannulas may be time-consuming and tedious. Agonists and antagonists may not be readily differentiated in binding studies. There are several advantages to our method. Our present method does not involve extensive surgery or manipulation and a large number of animals can be tested in a short period of time. In addition, agents that may not otherwise cross the blood brain barrier can be tested *in vivo*. In summary, this report presents a simple method which is of potential value in testing compounds with GABA-mimetic activities.

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