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On the Role of Endogenous GABA in the Forced Swimming Test in Rats

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BORSINI, F., A. MANCINELLI, V. D'ARANNO, S. EVANGELISTA AND A. MELI. *On the role of endogenous GABA in the forced swimming test in rats*. PHARMACOL BIOCHEM BEHAV 29(2) 275–279, 1988.—GABA content was reduced in the nucleus accumbens, cortex and brainstem of rats after 5 but not after 45, 120 min or 24 hr, from the termination of the pretest session. This reduction was not observed in rats performing on rotarod. Intraperitoneal AOAA (25 mg/kg; 24, 5 and 1 hr before the test), reduced at the same extent immobility time regardless whether the animals had been exposed to a pretest session. In pretested animals, reduction in immobility time produced by AOAA (25 mg/kg×3 times) was similar to that observed following 50 mg/kg, 5 hr before testing. This reduction was not antagonized by GABA antagonists bicuculline (2 mg/kg) or picrotoxin (2 mg/kg), given intraperitoneally 30 and 20 min before the test respectively. Intraperitoneal sodium valproate (200 or 400 mg/kg; 24, 5 and 1 hr before the test) and isoniazide (200 mg/kg) or 4-deoxypyridoxine (400 mg/kg), administered 1 or 1.5 hr before the test, were ineffective. AOAA (25 mg/kg×3 times) gave a similar increase in GABA levels to 50 mg/kg only once in the brainstem, nucleus accumbens and hypothalamus and a greater increase in the other brain areas. After 5 hr from single dosing, 25 mg/kg AOAA increased GABA levels less than 50 mg/kg AOAA in the brainstem, nucleus accumbens, frontal cortex and striatum, and increased it to same extent in the other areas. Sodium valproate (400 mg/kg×3 times) increased GABA levels in all brain areas, except hippocampus, although to a lesser extent than AOAA.

Animal model of depression Forced swimming test Endogenous GABA GABAergic drugs

THE forced swimming test (FST) has been suggested as a laboratory model of depression [21]. It consists in forcing a rat to stay afloat in a confined space. After an initial period of vigorous activity, the animal remains immobile (pretest). On the subsequent immersion (test), the onset of immobility is much more rapid. Antidepressants [21] as well as GABAergic agonists [5] reduce the time of immobility during the test session while picrotoxin, an agent interfering with GABA transmission, antagonizes the anti-immobility effect of some antidepressants [6]. Since GABAergic activity appears to be reduced in depressive phenomena [9, 11, 14, 20] and GABAergic compounds exert antidepressant activity in man [7,16], we thought it worthwhile to investigate the role of endogenous GABA on animal performance in this test.

The experiments aimed to assess whether or not: (a) the pretest would modify brain GABA levels and (b) drugs increasing or decreasing endogenous GABA levels would modify animal behavior during the test session. Since no information is available on brain areas in which GABA might have a role in swimming performance of the rat, GABA levels were determined in various brain regions.

METHOD

Animals

Male CD-COBS rats (Charles River, Italy), 160–190 g, were housed 4–5 to a cage, at constant temperature (22±1°C) and relative humidity (60%), with food and water ad lib, and with 12 hr light-dark cycle (light on: 6:00 a.m.). Each experimental group consisted of 7–8 animals and was chosen by means of a completely randomized schedule [3].

Forced Swimming Test

Rats were individually placed in Plexiglas cylinders (40 cm height, 18 cm i.d.) containing water (15 cm depth) maintained at 25±1°C ('pretest group'). Other animals were handled but not subjected to the pretest session ('non-pretest group'). After 15 min they were dried under a lamp for 30 min. The following day, the animals were placed in the cylinders for 5 min. A rat was judged to be immobile when it remained floating in the water making only those movements necessary to keep its head above water. The total duration of immobility during 5 min was recorded by an observer unaware of the treatment. Experiments were carried out between 1 and 5 p.m.

Measurement of GABA Levels

Since the most commonly used methods for immediate inactivation of post-mortem processes in the brain (rapid freezing or microwave irradiation) are not well suited to dissection of regional brain areas (see [23]), in order to prevent post-mortem increase in brain GABA levels, 100 mg/kg of 3-mercaptopropionic acid (an inhibitor of glutamic acid de-

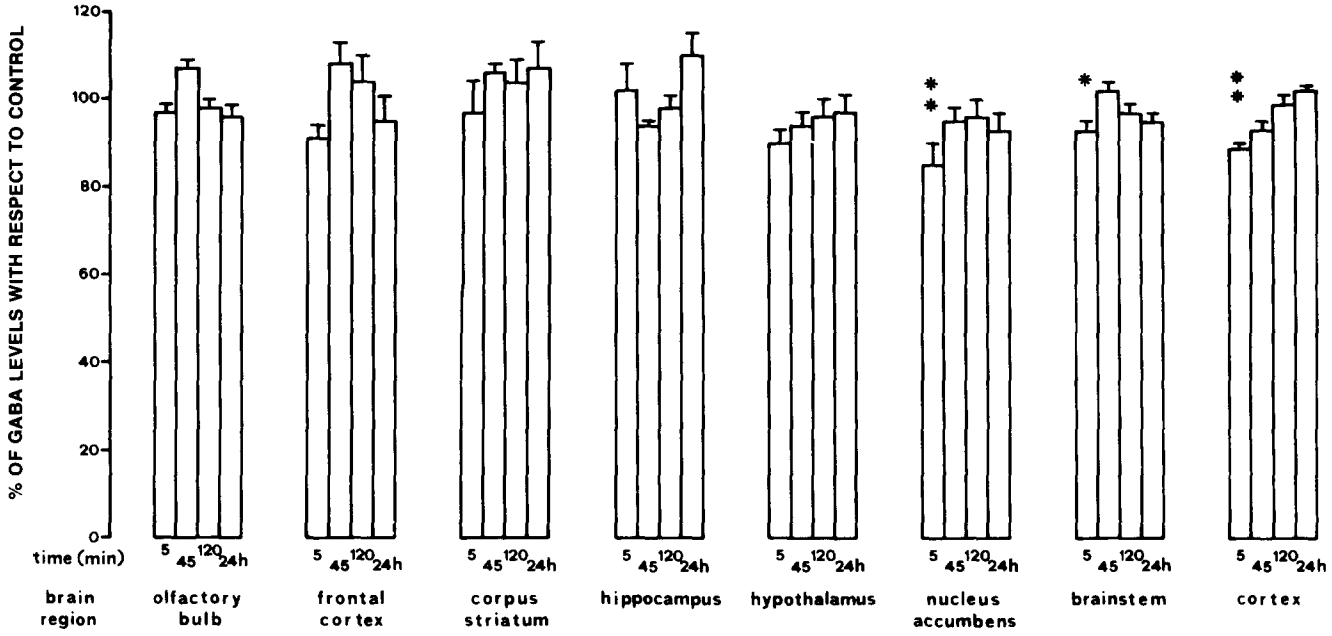

FIG. 1. Percent of GABA levels in different rat brain regions at 5, 45, 120 min and 24 hr after 15 min swimming. Columns represent means±s.e. of 7–8 rats. All animals were injected intraperitoneally with 100 mg/kg 3-mercaptopropionic acid 2.5 min before sacrifice. *$p<0.05$; **$p<0.01$ vs. respective control.

carboxylase) were administered intraperitoneally 2.5 min before killing by decapitation [18,23]. The brain was quickly removed and placed on an ice-cold Petri-dish. Various brain regions such as olfactory bulb, frontal cortex, corpus striatum, hippocampus, nucleus accumbens and the brainstem without the hypothalamus were dissected according to Glowinski and Iversen [10] and stored at −20°C until analysis. In experiments described in Fig. 1, the brainstem and the entire cortex were dissected out from the brain of one rat while the remaining regions were collected from another animal. The brain bilateral structures were pooled together. GABA levels were assayed using high-performance liquid chromatography (HPLC) procedure according to Zecca *et al.* [24], with minor modifications. Briefly, the brain regions were homogenized by means of a Politron homogenizer (brainstem and cortex) or sonicated by a Sonifer (all other brain regions) in 1.0 ml ethanol acidifed with 0.1% of 35% HCl containing valine as internal standard. Homogenates were centrifuged at 10.000 RPM at 0°C for 15 min. Aliquots (50–100 μl) of supernatant were removed and dried under nitrogen stream at 50°C. Residues were added 50 μl of 0.84% sodium bicarbonate solution and derivatized by reaction with 100 μl dansyl chloride solution (0.08% anhydrous acetone) and heated for 15 min at 80°C. After evaporation, 100 μl of 0.84% sodium bicarbonate solution were added to the residue and 20 μl were injected into the chromatograph. These values were plotted against a standard curve prepared by pooling supernatant samples from each brain region, containing known quantities of GABA and valine, processed as above and assayed in parallel with tissue samples. GABA and valine were determined by reverse-phase HPLC using a 40 mm perisorb RP-18 (30–40 μm) guard column coupled to a 250 mm ultrasphere ODS 5 μm column (Beckman Instr., CA, USA). The liquid chromatographic system consisted of a Beckman model 114 pump, sample injection valve Mod. 210, with 20 μl loop, and U.V. detector Mod. 160 (Beckman). Separation was performed by using an isocratic solvent system consisting of a water-acetonitrile (65/35) mixture containing 0.13% of 83% phosphoric acid. The solvent flow rate was maintained constant at 1.4 ml/min and monitored at 254 nm. GABA and dansyl chloride were obtained from Sigma (St. Louis, MO, USA). Valine and other chemicals were of analytical grade (Merck, Darmstadt, West Germany).

TABLE 1

WEIGHT OF BRAIN AREAS AND THEIR GABA CONTENT IN CONTROL RATS

Brain Areas	Weight (mg)	GABA Levels (μg/g tissue)
Total cortex	680 ± 7	192 ± 7
Frontal cortex	72 ± 2	186 ± 4
Olfactory bulb	67 ± 1	392 ± 5
Hypothalamus	42 ± 2	471 ± 11
Hippocampus	108 ± 1	216 ± 5
Nucleus accumbens	28 ± 1	380 ± 6
Striatum	110 ± 1	233 ± 5
Brainstem	447 ± 4	239 ± 3
Brainstem without hypothalamus	403 ± 7	227 ± 5

Values represent mean ± s.e.

Drug Treatment

Drugs were injected intraperitoneally as follows: aminooxyacetic acid (AOAA) 25 and 50 mg/kg either once at

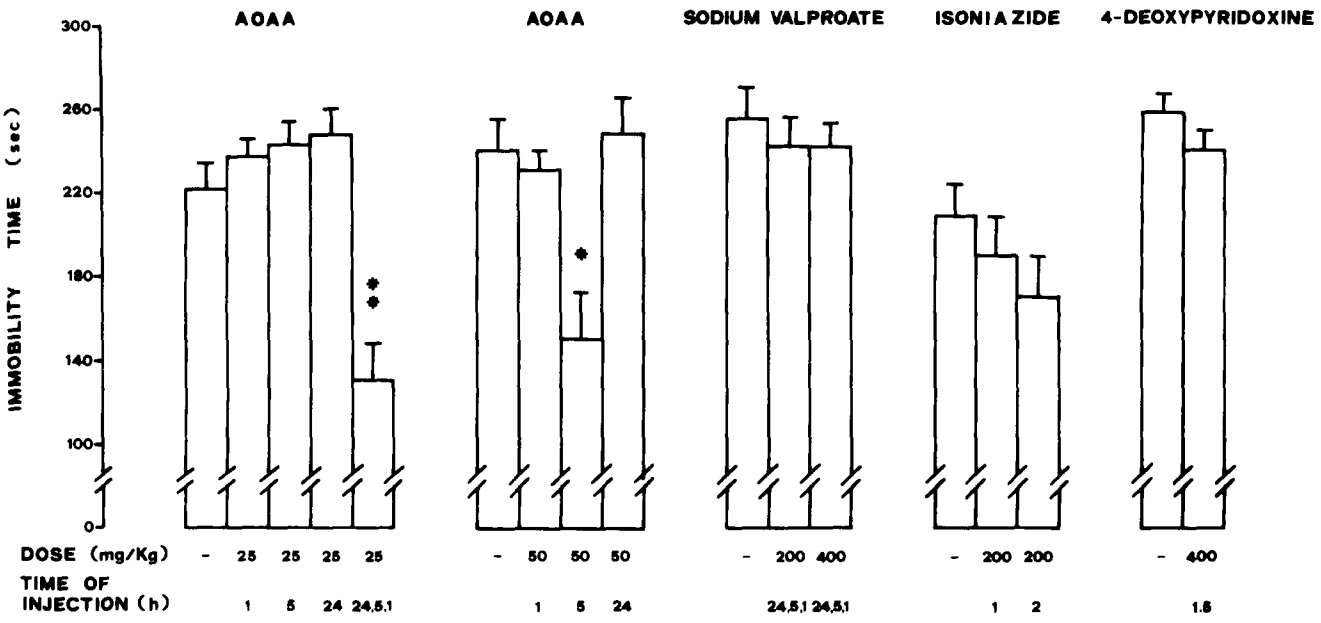

FIG. 2. Effect of aminooxyacetic acid (AOAA), sodium valproate, isoniazide and 4-deoxypyridoxine on immobility time. Columns represent mean±s.e. of 7–8 rats. All drugs were injected intraperitoneally. They were administered three times (24, 5 and 1 hr) or once (24, 5 or 1 hr) before the test. *$p<0.05$; **$p<0.01$ vs. respective control.

TABLE 2

EFFECT OF 25 mg/kg AOAA ON IMMOBILITY TIME OF RATS WHICH HAD BEEN (PRETEST GROUP) OR NOT SUBJECTED (NON-PRETEST GROUP) TO PRETEST SESSION

Experimental Group	Immobility Time (sec)	
	Vehicle	AOAA
Pretest Group	211 ± 12	150 ± 27†
Non-Pretest Group	174 ± 10*	90 ± 20 ns

Values represent mean ± s.e. from 8 rats.

AOAA was administered intraperitoneally 24, 5 and 1 hr before the test.

ns=not significant (ANOVA 2×2).

Tukey's test: *$p<0.05$; †$p<0.01$ vs. vehicle-pretest group.

24, 5 or 1 hr or three times (24, 5 and 1 hr) before the test or sacrifice; sodium valproate 200 and 400 mg/kg three times (24, 5 and 1 hr) before the test or sacrifice; isoniazide 200 mg/kg and 4-deoxypiridoxine 400 mg/kg only once 1 or 2 hr and 1.5 hr before the test, respectively.

Intraperitoneal picrotoxin (2 mg/kg) and bicuculline (2 mg/kg) were administered 20 and 30 min before the test respectively [5].

Drugs and Sources

Aminooxyacetic acid hemihydrochloride, 4-deoxypyridoxine hydrochloride, picrotoxin, 3-mercaptopropionic acid (Sigma, St. Louis, MO, USA) and isoniazide (Piam, Genova, Italy) were dissolved in distilled water. Sodium valproate was given as Depakin® (Sigma Tau, Roma, Italy). Bicuculline (Serva, Heidelberg, West Germany) was dissolved in distilled water by adding a few drops of 1 N HCl.

Control group received 5 ml/kg vehicle.

Statistics

Biochemical results were analysed by two-tailed Student's t-test. Behavioral data were analysed by two-tailed Dunnett's test or by factorial analysis of variance followed by Tukey's test.

RESULTS

The wet weight of several brain regions and their GABA content in control animals are shown in Table 1.

Figures relative to GABA content in brain regions at various times after termination of the pretest session are shown in Fig. 1. Five min after pretest, a significant reduction in GABA levels was observed in the nucleus accumbens ($p<0.01$), cortex ($p<0.01$) and brainstem ($p<0.05$). A similar but not significant reduction was observed in the hypothalamus and frontal cortex. No differences in GABA levels were observed in any brain areas 45 min, 2 or 24 hr after pretest. In order to ascertain whether changes in GABA levels were ascribable to physical exercise, GABA concentrations were determined in the cortex, nucleus accumbens and brainstem of rats forced to run on a rotarod (7 cm diameter; 10 rev/min) for a 15 min period and sacrificed 5 min later. GABA values (μg/g tissue) were (mean±s.e.)=cortex: 184±6 and 177±2; nucleus accumbens: 475±13 and 441±28; brainstem: 218±4 and 209±6, in controls and rats performing on a rotarod respectively. No difference resulted statistically different.

AOAA (25 mg/kg×3 times) reduced at the same extent immobility time regardless as to whether or not the animals

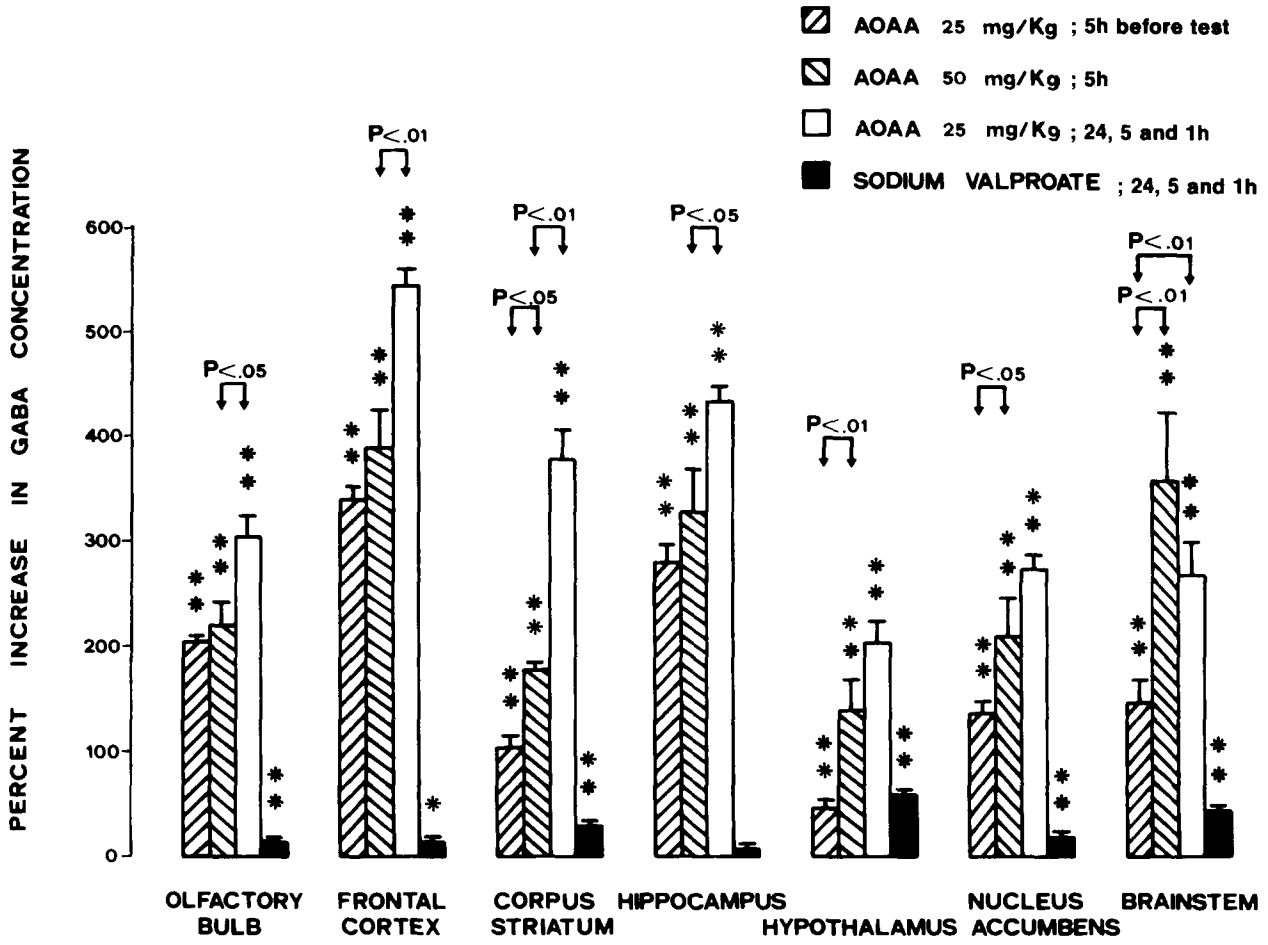

FIG. 3. Percent increase in regional GABA levels following administration with aminooxyacetic acid (AOAA) or sodium valproate (400 mg/kg). Columns represent mean±s.e. of 7–8 rats. The drugs were injected intraperitoneally. *$p<0.05$; **$p<0.01$ vs. respective control.

TABLE 3

EFFECT OF BICUCULLINE (2 mg/kg) AND PICROTOXIN (2 mg/kg) ON THE REDUCTION OF IMMOBILITY TIME INDUCED BY AOAA (25 mg/kg)

	Immobility Time (sec)	
Treatment	Vehicle	AOAA
Vehicle	217 ± 16	140 ± 32*
Bicuculline	238 ± 12	110 ± 33 ns
Vehicle	255 ± 08	164 ± 36*
Picrotoxin	273 ± 06	114 ± 26 ns

Values represent mean ± s.e. from 8 rats.

AOAA was administered intraperitoneally 24, 5 and 1 hr before the test. Intraperitoneal bicuculline and picrotoxin were injected 30 and 20 min before the test respectively.

ANOVA (2×2): ns=not significant.

Tukey's test: *$p<0.01$ vs. respective vehicle group.

had been exposed to a pretest session (Table 2). In pretested animals, reduction in immobility time produced by AOAA (50 mg/kg, 5 hr before testing) was similar to that observed following 25 mg/kg×3 times (Fig. 2). Rats given AOAA 50 mg/kg×3 times were not tested due to marked sedation. Sodium valproate (200 and 400 mg/kg×3 times) and AOAA (25 mg/kg only once) as well as isoniazide (200 mg/kg) and 4-deoxypyridoxine (400 mg/kg) were ineffective (Fig. 2). The dose and time of injection for isoniazide and 4-deoxypyridoxine which we used have been reported to reduce GABA levels in brain regions [15]. Higher doses were not used because of convulsions.

Neither picrotoxin nor bicuculline reduced the anti-immobility effect of AOAA (25 mg/kg×3 times) (Table 3).

Sodium valproate (400 mg/kg×3 times) increased GABA levels in all areas, except the hippocampus, although to a lesser extent than AOAA (Fig. 3). Increase in GABA levels in the corpus striatum, hypothalamus, nucleus accumbens and brainstem but not in the olfactory bulb, frontal cortex and hippocampus induced by AOAA given 5 hr before sacrifice was dose-dependent. AOAA (25 mg/kg×3 times) was more effective in increasing GABA levels in all brain regions than when administered at the same dose only once. AOAA (25 mg/kg×3 times) was more effective than when given at 50 mg/kg 5 hr before sacrifice in increasing GABA levels in the olfactory bulb, frontal cortex, corpus striatum and hippocampus but not in the hypothalamus, nucleus accumbens and brainstem.

DISCUSSION

Reduction in GABA levels in the nucleus accumbens

brainstem and cortex, observed in rats 5 min after exposure to a pretest session, was not detectable in animals performing on a rotarod for the same length of time. Therefore, the particular environmental condition rather than physical activity might be held responsible for changes in GABA levels. This reduction was transient, since it was no longer evident at later times. Whether this reduction influences immobility behavior during the test session or is secondary to changes in other systems, reported to occur after the pretest session [13,19], needs further investigations. However, the fact that AOAA-induced reduction in immobility time was independent from rat exposure to a pretest session suggests what happens to GABA levels at pretest time does not influence AOAA-induced immobility behavior during test session.

It is interesting that AOAA, at doses reducing immobility time, markedly increased GABA levels in the same areas (nucleus accumbens, brainstem and hypothalamus) where a decrease had been observed after the pretest. However, the fact that AOAA anti-immobility effect was not prevented by picrotoxin or bicuculline, suggests that this phenomenon is independent from GABAergic stimulation of either chloride ionophore or GABA-A receptors [1,12]. Likewise it appears unlikely that stimulation of GABA-B receptors could be held responsible for this effect, since baclofen, a GABA-B agonist, does not alter immobility time [5]. This casts some doubts on the role of GABA in FST. This is also substantiated by the fact that sodium valproate, whose pharmacological effects appear to be dependent upon elevation in GABA levels [17], did not affect immobility time. Since AOAA, unlike sodium valproate, increases extraneuronal GABA concentration [8], the possibility of activation of other types of GABAergic sites cannot be excluded. Alternatively, the possibility that AOAA may act via non-GABAergic neurons must be also taken into account, since AOAA also influences the catecholaminergic system [2], whose role in FST has been well documented [4,22]. These findings, and the fact that the GABA-decreasing drugs isoniazide and 4-deoxypiridoxine did not affect immobility time, suggest that changes in GABA levels might not play a primary role in FST. However, the observation that muscimol and THIP, agonists at GABAergic receptors, reduce immobility time by acting through picrotoxin-sensitive site/s [5] indicates a possible involvement of GABAergic mechanisms. Therefore, the possibility that elevation in GABA levels may activate different receptors with antagonistic functions thus resulting in a null effect must be taken into account and deserves further investigations.

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