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Ketamine-Induced Rotation: Interaction with GABA-Transaminase Inhibitors and Picrotoxin

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MYSLOBODSKY, M. S., R. F. ACKERMANN, V. GOLOVCHINSKY AND J. ENGEL, JR. *Ketamine-induced rotation: Interaction with GABA-transaminase inhibitors and picrotoxin.* PHARMAC. BIOCHEM. BEHAV. 11(5) 483–486, 1979.—Ketamine in a dose of 100 mg/kg (IP) produced stereotypic behavior and vigorous rotation in adult male Sprague-Dawley rats. The first rotation phase, accompanied by head swinging, was short and terminated by the anesthetic phase which lasted 20–30 min. The second rotation phase began 1–3 min after the end of the anesthetic phase. A single dose of GABA-T inhibitors, γ-vinyl GABA (1200 mg/kg, IP) or γ-acetylenic GABA (100 mg/kg, IP) administered 4 hours prior to ketamine, shortened the first rotation phase, increased the anesthetic phase, changed the pattern of postanesthetic rotation and reduced total and net rotation scores. Picrotoxin (3 mg/kg) given 10 min prior to ketamine tended to act in the opposite direction although none of its effects reached statistical significance.

Rotation Ketamine GABA-T inhibitors Picrotoxin

KETAMINE (dl-2-(*O*-chlorophenyl)-2-methylamino-cyclohexanone hydrochloride) is a rapidly-acting anesthetic and analgesic agent which is known to induce post anesthetic mood changes, confusion, hallucinations and irrational behavior in some humans [4]. In rats, ketamine produces a variety of excitatory effects [27] including regular and highly stereotypic locomotor phenomena which, at anesthetic doses, develop at the very beginning of, and emergence from, the anesthetic state ([13]; Glick, Personal Communication).

It has been shown that in unlesioned rats, rotational behavior reflects intrinsic asymmetry within the dopaminergic nigrostriatal system [5]. Ketamine is known to interfere with brain monoamine metabolism [2, 6, 8] and to increase the rate of dopamine turnover in the striatum [28] which conforms with the suggestion that dopamine mediates ketamine-induced circling. Since mesolimbic and nigrostriatal dopamine neurons appear to be under inhibitory GABA-ergic control [10, 12, 23], one would anticipate that GABA-mimetics and GABA antagonists interfere with effects produced by ketamine. The present study sought a systematic assessment of the effects of recently synethesized GABA-transaminase (GABA-T) inhibitors, γ-vinyl GABA and γ-acetylenic GABA, and a GABA antagonist, picrotoxin, on ketamine anesthesia.

METHOD

Subjects and Materials

Experiments were performed on adult, male Sprague-Dawley rats (Simonsen) and maintained in a standard laboratory environment (one per cage) with food and water ad lib. Night/day cycle (12 hr of darkness/12 hr of light) was maintained by artificial lighting.

The following substances were used throughout the study: ketamine hydrochloride (Parke-Davis), picrotoxin (Abbott), γ-vinyl GABA (GVG) and γ-acetylenic GABA (GAG), both generously donated by Merrell. All drug doses are expressed as weights of their salts.

Procedure

Animals were randomly assigned to four experimental groups: (1) ketamine group, (2) GVG-pretreated group, (3) GAG-pretreated group, (4) picrotoxin-treated group.

Animals in the first group were injected with ketamine (100 mg/kg, IP) and placed in rotometer bowls where the assessment of the main stages of anesthesia was conducted along with the evaluation of the rotation directionality as described elsewhere [13].

In brief, due to a short first rotation period, circling was scored exclusively in the postanesthetic period which lasted about 20–30 min. It began shortly (1–3 min) after the end of the anesthetic phase and was scored until the first pause in circling, provided the rats exhibited normal (i.e., nonataxic) posture. The assessment of rotation was started at the first full turn and always continued for 15 min irrespective of the amount of pauses made by the animal. The rotation score was expressed as the mean (± standard error of mean) of full turns per 15 min in the preferred and neglected directions.

An independent analysis of rotation under saline (0.9%) was conducted with this group of subjects either several days before or after analysis of ketamine effects.

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TABLE 1

EFFECTS OF GABA-T INHIBITORS AND PICROTOXIN ON DURATION OF KETAMINE-INDUCED ROTATIONAL AND ANESTHETIC PHASES (MEAN ± SEM MIN)

Compounds (doses)	N	First Rotational phase	Anesthetic phase	Postanesthetic rotation
Ketamine (100 mg/kg)	8	3.4 ± 0.9	18.0 ± 8.0	21.8 ± 2.3
Picrotoxin (3 mg/kg) + Ketamine	5	1.6 ± 0.6	10.2 ± 3.9	21.4 ± 2.3
GVG (1200 mg/kg) + Ketamine	5	0.2 ± 0.2*	41.4 ± 4.1	40.2 ± 3.9*
GAG (100 mg/kg) + Ketamine	8	0.7 ± 0.5*	47.8 ± 6.9*	52.1 ± 3.3*

*Significantly different from Ketamine alone at $p < 0.05$.

Groups 2 and 3 were pretreated with GVG (1200 mg/kg, IP) or GAG (100 mg/kg, IP) 4 hr prior to ketamine injection (100 mg/kg, IP) due to the reportedly long latency of the effect of these compounds [19,20]. Picrotoxin is known to produce its effect with a latency of about 10 min [26]. Correspondingly, it was injected in a dose of 3 mg/kg (IP) 10 min prior to ketamine (100 mg/kg, IP) (Group 4). Duration of the rotation and anesthetic phases were assessed as described.

Statistical Treatment

Dunnett's test for multiple comparisons with a control mean [11] was used throughout the study with rejection region at $p<0.05$. Statistical tests were performed on log transformations of the raw data.

RESULTS

Ketamine in a dose of 100 mg/kg reliably causes anesthesia, preceded and followed by the periods of stereotypic head swinging and rotation [13]. The effects of the GABA-T inhibitors and picrotoxin on these stages are summarized in Tables 1 and 2. Table 1 demonstrates that GVG and GAG reduced the duration of pre-anesthetic rotation due to a faster development of the anesthetic phase. An increase of the latter was evident after both GVG and GAG pretreatment although only in the GVG case did this difference reach statistical significance.

GVG and GAG pretreated rats also had longer periods of postanesthetic rotation. However, this rotation was qualitatively different from rotation under ketamine alone. The posture of rotating rats was less asymmetric and the circling occurred in short bursts of several incomplete turns in both directions, followed by a prolonged interval of immobility. Correspondingly, in spite of an increase in duration of rotation period (Table 2) GVG and GAG reliably reduced total rotation score. In addition, net rotation (a difference between the rotation score in the preferred and non-preferred direction) was reduced, indicating a weakening of directionality preference after GVG and GAG pretreatment.

The GABA antagonist, picrotoxin, tended to reduce the duration of the preanesthetic rotation period, the duration of the anesthetic phase, and the rotation score in the postanesthetic rotation. None of these results proved to be statistically significant.

TABLE 2

EFFECTS OF CHEMICAL COMPOUNDS TESTED ON KETAMINE-INDUCED ROTATIONAL BEHAVIOR IN RATS*

Pretreatment (dose)	N	Test Compound (dose)	Total Rotation (full turns/ 15 min)	Net Rotation
None	10	Saline	3.7 ± 1.1	1.5 ± 0.4
None	10	Ketamine (100 mg/kg)	63.4 ± 11.2†	27.8 ± 6.8†
Picrotoxin	5	Ketamine (100 mg/kg)	38.4 ± 7.9†	23.2 ± 6.1†
GVG	5	Ketamine (100 mg/kg)	24.5 ± 3.0†‡	2.8 ± 0.6‡
GAG	5	Ketamine (100 mg/kg)	17.8 ± 4.4†‡	4.0 ± 0.8‡

*Each value represents the mean (± SEM).
†Significantly different from Saline at $p<0.05$ level.
‡Different from Ketamine at $p<0.05$ level.

DISCUSSION

The study supports previous observations ([13]; Glick, Personal Communcation) that ketamine-induced turning in circles is a reliable phenomenon which may be modulated, but not eliminated entirely, by compounds affecting GABA system. This rotation cannot be attributed solely to the animals' postanesthetic hyperactivity. The latter should have resulted in equal amounts of both clockwise and counter-clockwise circling; on the contrary, under ketamine alone, animals rotated predominantly in one direction. Our previous findings have demonstrated that for a given animal the directionality of rotation under ketamine was the same as that under amphetamine [13].

Amphetamine is known to stimulate dopamine receptor indirectly, by releasing the catecholamine neurotransmitter from presynaptic stores [24]. Ketamine is known to interfere with metabolism of several major transmitters, dopamine [6, 8, 28], norepinephrine [6, 8, 21,28] and serotonin [2, 8, 21, 25, 28]

The role of serotonin in ketamine-induced rotation seems to be least significant. p-Chlorophenylalanine (PCPA), which depletes the brain of 5-HT by inhibiting its synthesis, does not alter amphetamine-induced circling [7]. In addition, the changes in serotonin metabolism have been noted with a delay of at least 3 hr after the ketamine administration [25], when the locomotion of animals has returned to normal.

Recent biochemical studies have demonstrated that ketamine [21] and its parent compound, phencyclidine [22], act as catecholamine-releasing agents and as competitive inhibitors of the high affinity transport system responsible for uptake of norepinephrine. Although norepinephrine is involved in rotation of rodents [1], the rotation is known to ultimately depend upon the asymmetry of striatal dopamine receptor [5,23].

In addition, ketamine (50 mg/kg) has been shown to remarkably reduce whole-brain dopamine content 30 min after injection [28]. Striatal homovanillic acid has been reported to increase by about 55% at 15 min and at 1 hr after administration of ketamine (100 mg/kg IP) [28], suggesting an acceleration of dopamine turnover at these times. The peak values of homovanillic acid seem to roughly correspond to the two phases of rotation observed in the present study. Unfortunately, comparisons of left-right hemisphere dopamine and homovanillic acid levels during ketamine anesthesia, apparently have not been conducted thus far. Homovanillic acid asymmetry, however, may be anticipated at least on the basis of: (a) previous observations that ketamine produces far more vigorous rotation than amphetamine [13]; (b) the report of Glick's group [5] that substances which increase rotation also increase the asymmetry of striatal dopamine content; (c) our present observation that a weakening of side preference occurred under GABA-T inhibitors (Table 2). The effect of GABA-T inhibitors requires special comment.

Injected systemically, GVG and GAG are known to remarkably increase GABA brain content [15]. Given the role of descending striatonigral and pallidonigral pathways [11,12] in regulation of the dopamine-containing nigrostriatal system, a major increase in striatal dopamine concentration and decrease in striatal dopamine turnover in response to GABA-T concentration is anticipated. In fact, GAG has reportedly caused a significant increase of the dopamine content in the striatum [15].

The rotational behavior of rodents may be controlled by more than one system. Some writers [10,16] argue that the imbalance of the nigrostriatal system creates only asymmetry of posture but does not cause locomotion. The latter is displayed provided the mesolimbic dopamine receptors are activated [9] and the asymmetry in nigrostriatal system provides a prepotent directionality vector which "channels" locomotion into circling [16]. Our observations indicate that the ability of GVG and GAG pretreated animals to display asymmetric posture is reduced, which is reflected by the reduction of the side preference and decrease of the amount of full turns and net rotation scores. Importantly, GAG reportedly does not have a significant effect on the concentration of dopamine in the nucleus accumbens [15]. Therefore, the increase in duration of the anesthetic phase and impairment of the rotation pattern after GVG and GAG pretreatment is consistent with the assumption that the ketamine acts via the dopamine nigrostriatal system.

Unexpectedly, the GABA antagonist, picrotoxin, did not reliably antagonize ketamine effects although it tended to act in the anticipated direction. It should be remembered that unlike GABA-T inhibitors, the effects of which were detectable at least 24 hr after administration [14], the half-life of a single convulsant dose of picrotoxin is about 40 min [25]. The relatively short duration of its action could make picrotoxin marginally effective at the time when the animals recover from the anesthetic phase and begin to circle. Higher doses of picrotoxin produce convulsions and, therefore, have not been used in the present study.

The induction of stereotyped behavior produced by a variety of stimulants has been considered an experimental model of psychotic behavior [18]. Ketamine is a psychotomimetic agent. Like phencyclidine, it has found its way onto the illicit drug market under the name of "green". Some writers have suggested that abnormal states induced by both compounds model rather accurately some aspects of schizophrenia [17]. In view of this evidence, the ability of GVG and GAG to interfere with ketamine-induced rotation suggests that these GABA-T inhibitors may have an antipsychotic effect. In fact, antipsychotic effects of GABA analogue, baclofen, have been noted [3].

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REFERENCES

1. Costall, B. and R. J. Naylor. Stereotyped and circling behaviour induced by dopaminergic agonists after lesions of the midbrain raphé nuclei. *Eur. J. Pharmac.* **29:** 206–222, 1974.
2. Fahim, I., M. Ismael and O. H. Osman. Role of 5-hydroxytryptamine in Ketamine-induced hypothermia in the rat. *Br. J. Pharmac.* **48:** 570–576, 1973.
3. Frederiksen, P. K. Baclofen in the treatment of Schizophrenia. *Lancet* **1:** 702, 1975.
4. Garfield, J. M., M. C. Frances, B. Garfield and J. G. Stone. A comparison of psychologic responses to ketamine and thiopental-nitrous oxide—Halothane anesthesia. *Anesthesiology* **36:** 329–338, 1972.
5. Glick, S. D., T. P. Jerussi and B. Zimmerberg. Behavioral and neuropharmacological correlates of nigrostriatal asymmetry in rats. In: *Laterization in the Nervous System*, edited by S. Harnad. New York: Academic Press, 1977, pp. 213–249.
6. Glisson, S. N., A. A. El-Etr and B. C. Bloor. The effect of Ketamine upon norepinephrine and dopamine levels in rabbit brain parts. *Naunyn-Schmiedberg's Arch. Pharmac.* **295:** 149–152, 1976.
7. Green, A. R. and P. H. Kelly.Evidence concerning the involvement of 5-hydroxytryptamine in the locomotor activity produced by amphetamine or tranylcypromine plus L-DOPA. *Br. J. Pharm.* **57:** 141–147, 1976.
8. Kari, H. P., P. P. Davidson, H. H. Kohl and M. M. Kochher. Effects of Ketamine on brain monoamine levels in rats. *Res. commun. Chem. Pathol. Pharmac.* **20:** 475–488, 1978.
9. Kelly, P. H. and K. H. Moore. Mesolimbic dopaminergic neurons in the rotational model of nigrostriatal function. *Nature* **263:** 695–696, 1976.
10. Kelly, P. H. and K. E. Moore. Dopamine concentrations in the rat brain following injections into the substantia nigra of baclofen γ-aminobutyric acid, γ-hydroxybutyric acid, apomorphine and amphetamine. *Neuropharmacology* **17:**169–174, 1978.

11. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*. Belmont, CA: Brooks/Cole, 1968.
12. McGeer, P. L. and E. G. McGeer. The GABA system and function of the basal ganglia: Huntington's disease. In: *GABA in Nervous System Function*, edited by E. Roberts, T. N. Chase and D. B. Tower. New York: Raven Press, 1976, pp. 287–295.
13. Myslobodsky, M., R. F. Ackermann, R. Mansour and V. Golovchinsky. Ketamine-induced rotation and its interactions with naloxone in rats. *Brain Res.* **172:** 191–196, 1979.
14. Myslobodsky, M. S., R. F. Ackermann and J. Engle, Jr. Effects of γ-acetylenic GABA and γ-vinyl GABA on metrazol-activated, and kindled seizures. *Pharmac. Biochem. Behav.* **11:** 265–271, 1979.
15. Palfeyman, M. G., S. Huot, B. Lippert and P. J. Schechter. The effect of γ-acetylenic GABA, an enzyme-activated irreversible inhibitor of GABA-transaminase on dopamine pathways of the extrapyramidal and limbic systems. *Eur. J. Pharmac.* **50:** 325–336, 1978.
16. Pycock, C. J. and C. D. Marsden. The rotating rodent: A two component system? *Eur. J. Pharmac.* **47:** 167–175, 1978.
17. Petracek, F. J., J. H. Biel, B. Bopp and B. D. Mitchell. Chemistry and structure-activity relationships of psychotropic drugs. In: *Principles of Psychopharmacology*, 2nd edition, edited by W. G. Clark and J. del Giudice. New York: Academic Press, 1978, pp. 133–168.
18. Randrup, A. and I. Munkvad. Biochemical, anatomical and physiological investigations of stereotyped behavior induced by amphetamines. In: *Amphetamines and Related Compounds*, edited by K. Costa and S. Garattini. New York: Raven Press, 1970, p. 695.
19. Schechter, P. J., Y. Tranier, M. J. Jung and A. Sjoerdsma. Antiseizure activity of γ-aminobutyric acid: a catalytic irreversible inhibitor of γ-aminobutyric acid transaminase. *J. Pharmac. exp. Ther.* **201:** 606–612, 1977.
20. Schechter, P. J., Y. Tranier, M. J. Jung and P. Bohlen. Audiogenic seizure protection by elevated brain GABA concentration in mice: effects of γ-acetylenic GABA and γ-vinyl GABA, two irreversible GABA-T inhibitors. *Eur. J. Pharmac.* **45:** 319–328, 1977.
21. Smith, P. J. and A. J. Azzaro. The effect of Ketamine HCl on synaptosomal high affinity transport systems for norepinephrine, serotonin and γ-aminobutyric acid in rat brain. *Pharmacologist* **16:** 325, 1974 (Abstr.).
22. Smith, R. C., H. Y. Meltzer, R. C. Arora and J. M. Davis. Effects of phencyclidine on (^{3}H) catecholamine and (^{3}H) serotonin uptake in synaptosomal preparations from rat brain. *Biochem. Pharmac.* **26:** 1435–1439, 1977.
23. Stevens, J., K. Wilson and W. Foote. GABA blockade, dopamine and schizophrenia: experimental studies in the cat. *Psychopharmacologia* **39:** 105–119, 1974.
24. Ungerstedt, U. Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. *Acta Physiol. Scand.* Suppl. **367:** 49–68, 1971.
25. Vargiu, L., E. Stefanini, C. Musinu and G. Saba. Possible role of brain serotonin in the central effects of Ketamine. *Neuropharmacology* **17:** 405–408, 1978.
26. Wang, S. C. and J. W. Ward. Analeptics. *Pharmac. Ther. B* **3:** 123–165, 1977.
27. Winters, W. D. Epilepsy or anesthesia with ketamine. *Anesthesiology* **36:** 309–312, 1972.
28. Ylitalo, P., L. Saarnivaara and L. Ahtee. Effect of ketamine anesthesia on the content of monoamines and their metabolites in the rat's brain. *Acta Aneasth. Scand.* **20:** 216–220, 1976.