Pharmacology Biochemistry & Behavior, Vol. 15, pp. 545–549, 1981. Printed in the U.S.A.

(RS)-α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid: Wet Dog Shakes, Catalepsy and Body Temperature Changes in Rats

WALDEMAR TURSKI, LECHOSŁAW TURSKI, STANISŁAW J. CZUCZWAR AND ZDZISŁAW KLEINROK

Department of Pharmacology, Institute of Clinical Pathology, Medical School, Jaczewskiego 8, PL-20-090 Lublin, Poland

Received 8 May 1981

TURSKI, W., L. TURSKI, S. J. CZUCZWAR AND Z. KLEINROK. *(RS)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid: Wet dog shakes, catalepsy and body temperature changes in rats.* PHARMAC. BIOCHEM. BEHAV. 15(4) 545–549, 1981.—(RS)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was microinjected into the lateral brain ventricle of conscious rats in order to evaluate its pharmacological effects. Microinjection (5 μl) were made unilaterally and the effects of AMPA were assessed for 6 hr. AMPA produced generalized myoclonic seizures, short lasting hypoactivity followed by hyperactivity and hyperthermia when low doses were injected (0.25–1.0 μg). When AMPA was injected at higher doses (1.5–5.0 μg) it produced generalized myoclonic seizures, a hypoactive phase and hypothermia rapidly followed by hyperthermia. As the seizure activity and hypoactive phase receded, AMPA at doses of less than 2.5 μg produced hyperactivity and wet dog shakes in a dose-related manner. After receiving AMPA at doses of 2.5 and 5.0 μg, rats developed transient catalepsy. High quantities (5.0 μg) evoked a spectrum of generalized convulsive seizures lasting for 2–3 hr (1 seizure every 15 min). Biochemical assays showed that AMPA had complex effects on brain aminergic systems. AMPA decreased brain NA while brain DA concentration was slightly increased in a dose dependent manner. Moreover, AMPA increased brain 5-HT and 5-HIAA concentration in a dose- and time-related manner.

AMPA Wet dog shakes Catalepsy Hypothermia Hyperthermia Seizures

(RS)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) is a conformationally restricted structural analogue of ibotenic acid first synthesized by Honore and Lauridsen [10]. It has been shown to excite spinal interneurons and Renshaw cells in cats when applied microelectrophoretically [15]. The excitatory effect of AMPA was reversibly antagonized by glutamic acid diethyl ester, a putative glutamate antagonist [7] whereas D-α-aminoadipic acid, a potent aspartate antagonist [4] had no antagonistic effect [15]. These findings demonstrated that AMPA-induced neuronal excitation was achieved by activating glutamate receptors. Kainic acid, a naturally occuring structural analogue of glutamate isolated from Digenea simplex [18] also exerts a powerful excitatory effect on central neurons [23]. It is known that intracerebroventricularly administered kainic acid elicits in rats a characteristic pattern of behavioral abnormalities including hypothermia followed by hyperthermia [25], wet dog shakes [13,16] and seizures [1,19]. However, kainic acid seems to interact with only a small number of receptor sites for L-glutamic acid [8, 17, 22] and thus has insufficient specificity to be useful in the study of central glutamate receptors [15, 21, 22]. Since AMPA represents a new class of glutamate agonists and since detailed information concerning the behavioral consequences of the compound is not currently available, the present investigation was undertaken.

METHODS

Animals

Male Wistar rats weighing 180–200 g were used throughout the experiments. Animals were housed in colony cages with a natural light-dark cycle. Standard rat chow pellets (Murigran®) and tap water were continuously available. Rats were randomly assigned to experimental groups. Pharmacological testing was done between 10.00 a.m. and 5.00 p.m.

Procedure of AMPA Administration

(RS)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid was dissolved in an equimolar volume of 1 M hydrochloric acid and brought to the final volume with phosphate buffered saline (pH 7.35) and injected intracerebroventricularly with a Hamilton microsyringe (type 701 N) in a volume of 5 μl at doses of 0.25, 0.5, 1.0, 1.5, 2.5 and 5.0 mg, according to Herman [9] and Kleinrok and Turski [13]. A point 1.3

mm lateral and 1.5 mm posterior from bregma was the site of skull puncture. Under light ether narcosis, 24 hr before intracerebroventricular injection a craniotomy hole was drilled above the lateral ventricle. The injection needle fitted with a nylon cuff to attain a depth of 4.2 mm was placed perpendicular to the surface of the skull. Injection was made over 15 sec and the needle was maintained in position for an additional 15 sec. The control rats received the same volume of solvent by the same route. Intracerebral injections were made unilaterally.

Temperature Measurements

The body temperature of rats was measured by means of a thermistor thermometer (Ellab, Copenhagen, Denmark), the probe being inserted into the rectum to a depth of 30 mm. The mean of three preliminary measurements taken at 30 min intervals for 1 hr. served as the reference point for determination of the temperature changes after intracerebroventricular injection of AMPA. Body temperature changes were presented as differences (Δt) between the mean temperature before and after AMPA administration. Environmental temperature was kept constant at 21±1°C.

Assessment of Wet Dog Shake Behavior

Wet dog shakes were counted 30 min after intracerebroventricular administration of AMPA in 10 min periods over a duration of 2.5 hr. Rats were separately placed in Plexiglas cages (25 × 15 × 10 cm). Experiments were carried out in a well-lighted room maintained at 21±1°C.

Catalepsy

Catalepsy was measured by placing both forelegs on a steel bar 10 cm high for 15, 30 and 45 min after AMPA administration. If the rat remained in this position for at least 30 sec, the animal was considered cataleptic.

Biochemistry

For the measurement of brain catecholamine levels, the brains were homogenized in ice-cold 0.4 N perchloric acid and the homogenate was centrifuged for 10 min at 14,000 G. The amines were assayed spectrofluorometrically following the method of Chang [3] as modified by Brodie *et al.* [2]). Recoveries for the whole procedure were respectively 78.1% and 88.2% for NA and DA. For the determination of brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations, the brains were homogenized in ice-cold acidified n-butanol and the homogenate was centrifuged for 10 min at 14,000 G. The 5-HT and 5-HIAA were determined spectrofluorometrically following the method of Curzon and Green [6]. Recoveries for the whole procedure were respectively 82.8% and 93.2% for 5-HT and 5-HIAA.

Statistics

The data collected from behavioral and biochemical experiments were statistically analyzed by means of Student's *t*-test.

RESULTS

Behavior

After receiving an unilateral intracerebroventricular injection of AMPA rats underwent a series of behavioral abnormalities. For 10–60 sec after injection of AMPA at all doses tested, the rats exhibited generalized clonic seizures and lost their righting reflex. After AMPA at doses of less than 2.5 μg the animals became hypoactive for at least 1 hr and after receiving AMPA at doses of 2.5 and 5.0 μg rats developed cataleptic behavior lasting from 15–45 min after injection (Table 1). As the dramatic seizure activity and hypoactive phase receded, the rats exhibited at doses of less than 2.5 μg an increase in activity lasting up to 2–3 hr after injection. Between 30–60 min and 3 hr after injection wet dog shakes predominated among other behaviors. Wet dog shakes did not occur at doses of less than 1.0 μg and greater than 2.5 μg of AMPA. One hr postinjectioin animals receiving AMPA at doses of greater than 2.5 μg developed ipsilateral rotating behavior with episodic dystonic turning toward the side ipsilateral to injection or axial rotating with occasional contralateral rotations lasting up to 3 hr. Administration of AMPA at a dose of 5.0 μg produced frequent generalized convulsive seizures (1 every 15 min) which appeared after 1–2 hr and lasted for 3–5 hr. Moreover, animals frequently showed teeth chattering, jerks, and sometimes salivation. Straub phenomena and a squirrel-like upright posture.

TABLE 1

PERCENTAGE OF ANIMALS RESPONDING EITHER TO SEIZURES OR PRODUCTION OF CATALEPSY OR BOTH AFTER INTRACEREBROVENTRICULAR INJECTION OF (RS)-α-AMINO-3-HYDROXY-6-METHYL-4-ISOXAZOLEPROPIONIC ACID (AMPA)

	Seizures	Catalepsy		
		15 min	30 min	45 min
Control	0 (0/7)		0 (0/7)	
AMPA (μg)				
0.25	56.3 (9/16)		0 (0/16)	
0.5	75.0 (12/16)		0 (0/16)	
1.0	83.3 (20/24)		0 (0/16)	
1.5	96.7 (29/30)	0/30	10.0 (3/30)	0/30
2.5	100.0 (34/34)	13/34	88.2 (30/34)	17/34
5.0	100.0 (18/18)	16/18	100.0 (18/18)	11/18

Body Temperature Changes

The time courses and changes in body temperature after the intraventricular injection of various doses of AMPA are shown in Fig. 1, A and B. AMPA (0.25–1.0 μg) administered into the lateral ventricle induced a hyperthermic response which reached its maximum in 60 min and persisted on this level up to 6 hr after injection (Fig. 1A). As shown in Fig. 1B, injection of AMPA at doses of greater than 1.5 μg resulted in a dose-dependent and biphasic effect on body temperature. High doses of AMPA (1.5–5.0 μg) initially induced hypothermia (at 30–45 min) and then temperature abruptly increased reaching basal level at 60–90 min and went even higher by 120 min after injection. This hyperthermic effect lasted more than 6 hr after the injection. The injection of saline did not significantly alter body temperature.

Wet Dog Shake Behavior

Wet dog shakes began 25–30 min after intracerebroventricular AMPA injection (Fig. 2). Number of wet dog shakes increased in a dose-dependent manner. The intensity of wet

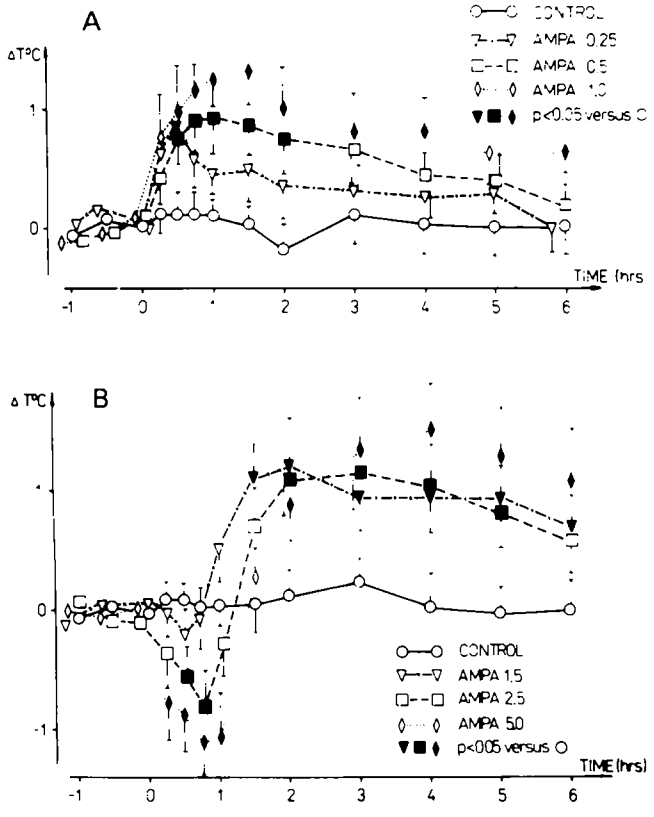

FIG. 1. A: Effect of (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) on body temperature of rats. Each point: mean of measurements in 8 animals. Vertical bars: standard deviations. A: Effect of low doses of AMPA (0.25–1.0) on body temperature of rats. (○——○) saline; (▽-×-▽)AMPA 0.25 μg; (□- -□)AMPA 0.5 μg;(◇···◇)AMPA 1.0 μg. Values of AMPA in all doses were compared with those of saline (filled triangles, squares and rhombs) $p<0.05$ for the entire period of observations. B: Effect of high doses of AMPA (1.5–5.0 μg) on body temperature of rats (○——○)saline; (▽-×-▽)AMPA 1.5 μg; (□——□)AMPA 2.5 μg; (◇···◇)AMPA 5.0 μg. Values of AMPA in all doses were compared with those of saline (filled triangles, squares and rhombs). $p<0.05$ for the entire period of observations.

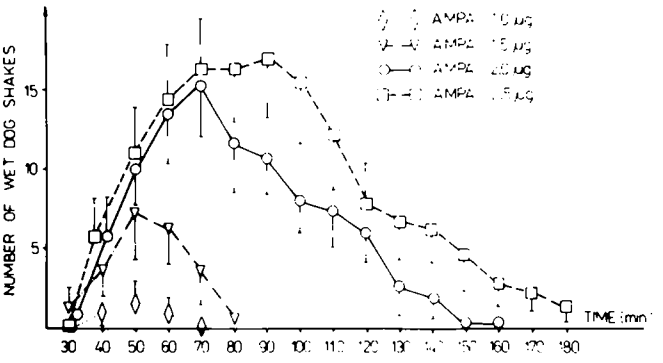

FIG. 2. The time course and dose-response effects of (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) given intracerbroventricularly on wet dog shake behavior in rats. Wet dog shakes were counted in 10 min periods. Each value represents mean (WDS number/10 min) ± SD of 8 animals.

dog shakes reached its maximum 70–90 min after the administration of the compound. AMPA-induced wet dog shakes gradually disappeared up to 3 hr after intracerebroventricular administration. Wet dog shakes did not occur in rats at doses of less than 1.0 μg and at doses of greater than 2.5 μg of AMPA. The injection of saline in control rats did not elicit wet dog shakes.

Cataleptsy

AMPA (2.5–5.0 μg) injected into the lateral brain ventricle induced a cataleptic response in 15 min which reached its maximum in 30 min and than abrubtly declined 45 to 60 min after injection.

Biochemistry

Injection of AMPA into the lateral brain ventricle induced a clear dose-dependent decrease in brain NA concentration. Moreover, AMPA at a dose of 1.0 μg consistently increased brain DA concentration, which reached a maximum at 2 hr and then gradually decreased up to 6 hr after injection. However, AMPA at a dose of 2.5 μg failed to significantly alter brain DA concentration. In contrast, AMPA considerably increased brain 5-HT and 5-HIAA concentration in a dose and time-related manner (Table 2).

DISCUSSION

Kainic acid, a structurally restricted analogue of L-glutamic acid, binds to brain membranes with high and low-affinity [17, 22, 24] and considerable regional distribution of kainic acid binding sites in brain tissue has been well documented [5,17]. Kainic acid, however, does not bind exclusively to synaptic receptors, and thus should not be used as a selective pharmacological tool for studying glutaminergic receptor events [21,22]. Neurochemical, morphological and behavioral effects of intracerebroventricular injections of kainic acid have been well documented [1, 12, 13, 19, 20, 26]. However, one must be very cautious in assuming that there is a critical connection between kainic acid produced behavioral, neurochemical and morphological phenomena and the physiological role of central glutaminergic receptors. From our present experiments it is apparent that there is a good correlation between acute behavioral effects of AMPA and kainic acid. However, some basic differences exist. As was mentioned in the introductory paragraph, intracerebroventricularly administered kainic acid produces a characteristic pattern of behavior in conscious rats: small quantities of the neurotoxin (0.05–0.1 μg) produce wet dog shakes [13,16], hypothermia rapidly followed by hyperthermia [25], seizure-like activity (the first generalized seizure after approximately 1–2 hr; [1,11]), and sometimes salivation, teeth chattering, Straub tail phenomenon and a squirrel-like upright position [1, 14, 19]; high quantities (0.2–0.5 μg) evoke a spectrum of generalized convulsive seizures (lasting for 2–3 hr) with a frequency of 1 convulsive attack every 5 min, hyperactivity, biting behavior, hyperthermia and sometimes wet dog shakes (unpublished observations). AMPA has been

TABLE 2

EFFECT OF (RS)-α-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPIONIC ACID (AMPA) ON MONOAMINE AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) CONCENTRATIONS IN WHOLE RAT BRAIN

	Noradrenaline	Dopamine	5-HT	5-HIAA
Control	0.541 ± 0.026	1.354 ± 0.036	0.569 ± 0.014	0.621 ± 0.018
AMPA 1.0				
1 hr	0.508 ± 0.027	1.565 ± 0.056†	0.607 ± 0.018	0.774 ± 0.034‡
2 hr	0.464 ± 0.023*	1.674 ± 0.049‡	0.711 ± 0.034†	0.953 ± 0.039‡
3 hr	0.472 ± 0.029	1.467 ± 0.055	0.692 ± 0.039†	0.939 ± 0.045‡
6 hr	0.483 ± 0.019	1.354 ± 0.026	0.625 ± 0.014*	0.809 ± 0.059†
AMPA 2.5				
1 hr	0.385 ± 0.029†	1.475 ± 0.059	0.817 ± 0.035‡	1.155 ± 0.041‡
2 hr	0.344 ± 0.022‡	1.286 ± 0.061	0.882 ± 0.033‡	1.425 ± 0.049‡
3 hr	0.332 ± 0.028‡	1.329 ± 0.037	0.943 ± 0.041‡	1.432 ± 0.036‡
6 hr	0.283 ± 0.009‡	1.349 ± 0.033	0.860 ± 0.037‡	1.381 ± 0.045‡

AMPA was injected intracerebroventicularly in doses of 1.0 and 2.5 μg. The animals were killed 1, 2, 3, or 6 hr after AMPA. Control rats received ICV the same volume of saline. Shown are the mean (μg.g $^{-1}$) ± SD of 8 determinations. Values were corrected according to the respective recoveries.

Statistical significances were calculated according to Student's *t*-test. Differs from the respective control: *$p<0.05$; †$p<0.01$; ‡$p<0.001$.

proposed to be a powerful and selective glutaminergic agonist, and it is structurally related to ibotenic acid [15]. However, our results show basic differences between the behavioral consequences of AMPA and kainic acid. In spite of induction of wet dog shakes and hypothermia followed by hyperthermia which may resemble the effect of kainic acid, immedicately after injection AMPA produces generalized myoclonic seizures and hypoactivity. The potent seizure activity of AMPA corresponds well with and resembles that evoked by intracerebroventricular ibotenic acid (unpublished observations) and not that of kainic acid. On the other hand, detailed information concerning the induction of wet dog shakes and changes in rat body temperature by intracerebroventricular ibotenic acid has not been available. Moreover, intracerebroventricularly administered kainic acid produces a considerable decrease in brain NA and a dose-related increase in brain 5-HIAA concentrations, while brain concentrations of DA and 5-HT remain unchanged [12,13]. In fact these findings fit well with currently reported biochemical consequences of intracerbral AMPA in rats. Summing up, the behavioral and biochemical pattern of abnormalities evoked in rats by AMPA resemble that of ibotenic acid (early seizures, hypoactivity, catalepsy) and that of kainic acid (wet dog shakes, changes in body temperature, distant seizures, disturbances in the brain aminergic systems). If AMPA represents a mixed complex of ibotenic and kainic acid-induced behavioral and biochemical consequences a question remains with respect to possible neurotoxic effects of the compound and further investigation is required before considering AMPA as a valuable tool for the study of the pharmacology and physiology of central glutamate receptors.

ACKNOWLEDGEMENTS

We gratefully acknowledge the generous gift of AMPA by Dr. Tage Honore, Royal Danish School of Pharmacy, Copenhagen, Denmark.

REFERENCES

1. Ben-Ari, Y., E. Tremblay, O. P. Ottersen and B. S. Meldrum. The role of epileptic activity in hippocampal and 'remote' cerebral lesions induced by kainic acid. *Brain Res.* **191**: 79–97, 1980.
2. Brodie, B. B., M. S. Corner, E. Costa and A. Dlabac. The role of brain serotonin in the mechanism of the central action of reserpine. *J. Pharmac. exp. Ther.* **152**: 340–349, 1966.
3. Chang, C. C. A sensitive method for spectrophotofluorometric assay of catecholamines. *J. Neuropharmac.* **3**: 643–649, 1964.
4. Collingride, G. L. and J. Davies. An evaluation of D-α-aminoadipate and D- (and DL-) -aminosuberate as selective antagonists of excitatory amino acids in the substantia nigra and mesencephalic reticular formation of the rat. *Neuropharmacology* **18**: 193–199, 1979.
5. Coyle, J. T., R. Zaczek, J. Slevin and J. Collins. Neuronal receptor sites for kainic acid–correlations with neurotoxicity. In: Glutamate as a neurotransmitter. *Adv. biochem. Psychopharmac.* **27**: 337–346, 1981.
6. Curzon, C. and A. R. Green. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of the brain. *Br. J. Pharmac.* **39**: 653–655, 1970.
7. Haldeman, S. and H. McLennan. The antagonistic action of glutamic acid diethylester towards amino-acid induced and synaptic excitations of central neurons. *Brain Res.* **45**: 419–425, 1972.
8. Hall, J. G., T. P. Hicks and H. McLennan. Kainic acid and glutamate receptor. *Neurosci. Lett.* **8**: 171–175, 1978.

9. Herman, Z. S. The effects of noradrenaline on rats' behaviour. *Psychopharmacologia* **16:** 369–374, 1970.
10. Honore, T. and J. Lauridsen. Structural analogues of ibotenic acid. Syntheses of 4-methyl-homoibotenic acid and AMPA, including the crystal structure of AMPA, monohydrate. *Acta chem. scand., B* **34:** 235–240, 1980.
11. Kleinrok, Z., S. J. Czuczwar, L. Turski. Prevention of kainic acid-induced seizure-like activity by antiepileptic drugs. *Pol J. Pharmac. Pharm.* **32:** 261–264, 1980.
12. Kleinrok, Z. and L. Turski. Biochemical consequences of kainic acid injections into the lateral brain ventricle in the rat. *Acta biochim. pol.* 1981, in press
13. Kleinrok, Z. and L. Turski. Kainic acid-induced wet dog shakes in rats. The relation to central neurotransmitters. *Naunyn-Schmiedeberg's Arch. Pharmac.* **314:** 37–46, 1980.
14. Kleinrok, Z., L. Turksi, M. Wawrzyniak and R. Cybulska. The locomotor and exploratory activities in rats after lesion of hippocampal pyramidal cells with kainic acid. *Pol. J. Pharmac. Pharm.* **32:** 625–637, 1980.
15. Krogsgaard-Larsen, P., T. Honore and J. J. Hansen. New class of glutamate agonist structurally related to ibotenic acid. *Nature* **284:** 64–66, 1980.
16. Lanthorn, T. and R. L. Isaacson, Studies of kainate-induced wet-dog shakes in the rat. *Life Sci.* **22:** 171–178, 1978.
17. London, E. D. and J. T. Coyle. Specific binding of [^{3}H] kainic acid to receptor sites in rat brain. *Molec. Pharmac.* **15:** 492–505, 1979.
18. Murayama, K., S. Morimura, Y. Nakamura and G. Sunagawa. Synthesis of pyrrolidine derivatives. (I) Synthesis of 2-decarboxykainic acid. *J. Pharm. Soc. Jap.* **85:** 130–142, 1965.
19. Nadler, J. V., B. W. Perry and C. W. Cotman. Intraventricular kainic acid preferentially destroys hippocampal pyramidal cells. *Nature* **271:** 676–677, 1978.
20. Nadler, J. V., B. W. Perry and C. W. Cotman. Preferential vulnerability of hippocampus to intraventricular kainic acid. In: *Kainic Acid as a Tool in Neurobiology*, edited by E. G. McGeer, J. W. Olney and P. L. McGeer. New York: Raven Press, 1978, pp. 219–237.
21. Peck, E. J. Receptors for amino acids. *A. Rev. Physiol.* **42:** 615–627, 1980.
22. Schwarcz, R. and K. Fuxe. ^{3}H-Kainic acid binding: Relevance for evaluating the neurotoxicity of kainic acid. *Life Sci.* **24:** 1471–1480, 1979.
23. Shinozaki, H. Discovery of novel actions of kainic acid and related compounds. In: *Kainic Acid as a Tool in Neurobiology*, edited by E. G. McGeer, J. W. Olney and P. L. McGeer. New York: Raven Press, 1978, pp. 17–35.
24. Simon, J. R., J. F. Contrera and M. J. Kuhar. Binding of [^{3}H] kainic acid, an analogue of L-glutamate, to brain membranes. *J. Neurochem.* **26:** 141–147, 1976.
25. Turski, L. and Z. Kleinrok. Effects of kainic acid on body temperature of rats: Role of catecholaminergic and serotonergic systems. *Psychopharmacology* **71:** 35–39, 1980.
26. Wuerthele, S. M., K. L. Lovell, M. Z. Jones and K. E. Moore. A histological study of kainic acid-induced lesions in the rat brain. *Brain Res.* **149:** 489–497, 1978.