Pharmacology Biochemistry & Behavior, Vol. 37, pp. 445–449. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Putrescine Decreases Exploration of a Black and White Maze

P. A. FERCHMIN[1] AND VESNA A. ETEROVIĆ

Department of Biochemistry School of Medicine, Universidad Central del Caribe
Call Box 60-327 Bayamon, Puerto Rico 00621-6032

Received 8 March 1990

FERCHMIN, P. A. AND V. A. ETEROVIĆ. *Putrescine decreases exploration of a black and white maze.* PHARMACOL BIOCHEM BEHAV **37**(3) 445–449, 1990.—The effect of putrescine and cyclohexylamine on rat cortical polyamine concentration and on behavior in a black and white maze was studied. The levels of polyamines in brain cortex were determined 15 min, 2, 4, and 6 hours after injection of putrescine (200 or 400 mg/kg) or cyclohexylamine (380 mg/kg). Putrescine concentration increased 6-fold 15 min after injection of putrescine followed by a decline during the next 6 hours. Cyclohexylamine increased putrescine concentration doubling it 4 hours after injection. Spermidine and spermine concentrations did not change after either putrescine or cyclohexylamine injection. Behavior was studied in the Greek cross maze which provides the choice to enter either white or black compartments. Putrescine 200 mg/kg decreased entries into white but not black compartments, while putrescine 400 mg/kg decreased entries into both. The effect of cyclohexylamine was similar to putrescine 400 mg/kg. The behavioral effect of each treatment was independent of the time between injection and testing for up to 6 hours, while the levels of putrescine changed during the same period. Therefore, behavior was not directly related to total cortical putrescine.

Putrescine Cyclohexylamine Exploratory behavior Rat brain cortex White-dark preference

THE polyamines putrescine, spermidine and spermine regulate a series of brain processes which in turn underlie certain behaviors. For example, the physiological phenomenon of long-term potentiation in the hippocampus is related to spatial learning (12). The establishment of LTP requires the participation of the N-methyl-D-aspartate receptor, phosphatidylinositol phosphates turnover, and activity of protein kinases (11), all of which are regulated by polyamines (15, 16, 18). It is therefore likely that polyamines regulate behavior, and that alteration in brain polyamine levels or metabolism will alter certain behaviors.

The relation between brain polyamines and behavior was not systematically studied. We have observed that polyamines are involved in experience-induced brain plasticity, and that injection of difluormethylornithine (DFMO), an inhibitor of putrescine synthesis, decreases the exploration of an enriched environment (7).

The present work was undertaken to determine whether an increase in brain putrescine level will affect the exploratory activity of a black and white maze (the Greek cross maze). This test measures the degree of preference for black over white compartments, which has been reported to depend on the emotional state of the animal (3, 6, 8). Putrescine was chosen to be studied first because of its low toxicity. Contrary to spermidine and spermine, putrescine has low toxicity. A single intraperitoneal injection of 60 mg/kg of spermine is fatal in mice causing death by nephrotoxicity within 6 days. Putrescine, 200 mg/kg, is not toxic even after five daily injections (17).

METHOD

Subjects

One hundred thirty-six Sprague-Dawley albino male rats bred in our colony were used for the behavioral study; they were distributed among five experiments as indicated in legends to Tables 1 to 3. Within 4 days after birth each litter was reduced to 8 pups, leaving the maximum amount of males. The animals were habituated to handling until 30-day-old when they were weaned. The experiments started when the animals were from 31 to 37 days of age. Seventy additional rats of the same strain, sex and ages were used for the determination of cortical polyamine levels. The legends to Figs. 1 to 3 indicate the numbers of rats used in every experiment.

Behavioral and Pharmacological Treatments

The exploratory activity was determined in the Greek cross maze. This cross-shaped maze consists of a central gray compartment communicated by openings in the wall with two black compartments on two opposite sides and with two white compartments on the remaining sides. The walls of the apparatus were 38 cm high, the size of each compartment was 21 × 21 cm and the openings were 6 cm high semicircles. To allow observation the maze did not have a ceiling.

Putrescine dihydrochloride and cyclohexylamine sulfate [about

[1]Requests for reprints should be addressed to P. A. Ferchmin, Department of Biochemistry, School of Medicine, UCCEM, HURRA, Call Box 60-327, Bayamon, PR 00621-6032.

the correct name of this compound see (1)] were purchased from Sigma Chemical Co. (St. Louis). The drugs were injected intraperitoneally as neutral solution. Pairs of littermates were injected with drug or saline solution and tested either 15 min, 2, 4, or 6 hours later. The order of testing (drug first or saline first) was alternated from pair to pair. To start the test the rat was placed in the central gray compartment of the Greek cross and the times of entries into black, white and gray compartments were recorded during 5 min by two observers. Entries were scored whenever a rat entered a compartment or introduced into it the head and both front paws.

Quantitative Brain Dissections

The method for separating the cerebral cortex from subcortical areas and the description that follows were adapted from Bennett and Rosenzweig (2). In brief, the whole brain is removed from the skull after cutting the paraflocculi, the optic nerves and the olfactory nerves. It is turned upside down and the olfactory bulbs and the olfactory tubercles are cut off and discarded. The ventral cortex is gently freed from the adjacent hypothalamus with a scalpel blade. The brain is then turned right side up again, and starting from the caudal end of the cortex, the choroid fissure is opened, exposing the internal capsule; this allows a clean separation of the cortex from the underlying subcortex. Anteriorly, where the internal capsule becomes continuous with the corona radiata, the cortical sample is separated from the underlying caudate nuclei by cutting through the internal capsule and continuing around the frontal surface of the caudate nucleus. The presence of the lateral ventricle allows for a natural separation of the hippocampus from the underlying diencephalon. Thus, our cerebral cortex sample also includes the corpus callosum, the hippocampus and the amygdaloid complex.

Determination of Polyamines

After dissection the brain cortex was weighed and frozen in liquid nitrogen. The frozen tissue samples were homogenized in perchloric acid and the polyamines were benzoylated and quantified by HPLC as reported previously (7).

Statistical Analysis

As explained above, we used a randomized block design superimposed on a factorial 2×2 design; one main factor was the presence or absence of drug, the other, the time elapsed between injection and testing. Both the behavioral and the neurochemical experiments followed the same design and were analyzed accordingly by a two-way analysis of variance for blocked data. All behavioral and neurochemical data were normally distributed and homoscedastic.

RESULTS

Behavioral Effects of Putrescine

Table 1 shows the effect of putrescine at 200 mg/kg on rat behavior in the Greek cross maze. Two experiments were done using, respectively, 12 and 16 pairs of drug-injected and saline-injected rats. The data for each experiment are presented separately and combined to illustrate the reproducibility between experiments.

Although animals were tested either 15 min, 2, 4, or 6 hours after injection, the statistical analysis did not reveal significant time effects (or a significant interaction between time and drug) in any of the experiments reported here. Therefore, the data from observations done at the different times after injection were pooled.

TABLE 1

EFFECT OF PUTRESCINE 200 mg/kg ON EXPLORATION OF THE GREEK CROSS MAZE

	Saline Mean ± SEM	Putrescine Mean ± SEM	% Diff.
Entries to white compartments			
Exp. 1	6.8 ± 0.5	5.0 ± 0.5	−26.2†
Exp. 2	6.0 ± 0.3	4.6 ± 0.5	−22.8†
Total	6.4 ± 0.3	4.8 ± 0.3	−24.5§
Entries to black compartments			
Exp. 1	9.7 ± 0.5	8.8 ± 0.7	−9.0
Exp. 2	8.8 ± 0.5	8.9 ± 0.7	1.5
Total	9.2 ± 0.4	8.9 ± 0.5	−3.5
Time spent in white compartments (sec)			
Exp. 1	74.4 ± 4.9	49.1 ± 8.0	−34.0†
Exp. 2	51.7 ± 3.3	40.8 ± 4.8	−21.2
Total	61.5 ± 3.5	44.3 ± 4.3	−27.8‡
Time spent in black compartments (sec)			
Exp. 1	110.3 ± 6.6	113.4 ± 10.9	2.8
Exp. 2	106.3 ± 9.0	144.0 ± 11.6	35.4†
Total	108.0 ± 5.8	130.8 ± 8.5	21.1*
Total number of entries			
Exp. 1	16.5 ± 0.9	13.9 ± 0.7	−15.9*
Exp. 2	14.8 ± 0.7	13.6 ± 1.0	−8.4
Total	15.5 ± 0.5	13.7 ± 0.6	−12.1*

Number of entries and time spent in sec during the 5 min of the test are presented. In Experiment 1, 12 putrescine vs. saline pairs were studied and in Experiment 2, 16 pairs. Statistical significance of the difference between drug- and saline-injected littermates, $p<$, is indicated by: *0.05; †0.025; ‡0.005; §0.001.

Entries into white compartments decreased significantly in subjects injected with putrescine 200 mg/kg [−24.5%, $F(1,48) = 12.1$, $p<0.001$], while entries into black compartments did not decrease significantly [−3.5%, $F(1,48) = 0.3$, $p =$ ns]. The time spent in white compartments declined [−27.8%, $F(1,48) = 9.2$, $p<0.005$], while the time spent in black compartments showed a reciprocal increase [21.1%, $F(1,48) = 5.2$, $p<0.027$].

An increase in the dose of putrescine from 200 to 400 mg/kg (Table 2) nearly doubled the decrease in the number of entries into white compartments [−39.5%, $F(1,16) = 8.1$, $p<0.025$], and introduced a significant decline in entries into black compartments [−30.9%, $F(1,16) = 6.8$, $p<0.025$]. The time spent in white [−4.9%, $F(1,16) = 0.0$, $p<0.875$] and in black [14.9%, $F(1,16) = 0.9$, $p<0.347$] compartments did not change significantly suggesting that the average time per visit increased. This increase, however, was also not significant (data not shown). Therefore, at 400 mg/kg, putrescine decreased both entries into white and into black compartments with only a marginally larger decrease in white over black entries.

Behavioral Effect of Cyclohexylamine

Cyclohexylamine (380 mg/kg, Table 3) decreased the number of entries into white [−49.0%, $F(1,48) = 21.6$, $p<0.001$] and

TABLE 2

EFFECT OF 400 mg/kg OF PUTRESCINE ON EXPLORATION OF THE GREEK CROSS MAZE

Saline Mean ± SEM	Putrescine Mean ± SEM	%Diff.
Entries to white compartments		
5.8 ± 0.6	3.5 ± 0.8	−39.5*
Entires to black compartments		
9.3 ± 0.7	6.4 ± 0.8	−30.9*
Time spent in white compartments (sec)		
63.0 ± 7.6	60.0 ± 17.8	−4.9
Time spent in black compartments (sec)		
143.9 ± 10.7	165.4 ± 22.2	14.9
Total number of entries		
15.0 ± 1.0	9.9 ± 1.4	−34.3†

Number of entries and time spent in sec during the 5 min of the test are presented. Twelve putrescine vs. saline pairs were studied. Statistical significance of the difference between drug- and saline-injected littermates, $p<$, is indicated by: *0.025; †0.01.

TABLE 3

EFFECT OF CYCLOHEXYLAMINE 380 mg/kg ON EXPLORATION OF THE GREEK CROSS MAZE

	Saline Mean ± SEM	Cyclohexylamine Mean ± SEM	% Diff.
Number of entries to white compartments			
Exp. 1	5.1 ± 0.5	3.9 ± 0.9	−24.0
Exp. 2	7.4 ± 0.5	2.8 ± 0.6	−58.0‡
Total	6.4 ± 0.4	3.3 ± 0.5	−49.0†
Number of entries to black compartments			
Exp. 1	10.4 ± 0.7	5.5 ± 1.0	−47.0†
Exp. 2	9.2 ± 0.7	3.5 ± 0.7	−61.0†
Total	9.7 ± 0.5	4.4 ± 0.6	−55.0‡
Time spent in white compartments (sec)			
Exp. 1	48.5 ± 6.4	47.7 ± 11.5	2.0
Exp. 2	94.6 ± 14.3	85.0 ± 23.9	−10.0
Total	74.9 ± 9.5	65.0 ± 14.7	−8.0
Time spent in black compartments (sec)			
Exp. 1	131.8 ± 6.6	104.7 ± 23.7	−21.0
Exp. 2	100.7 ± 12.2	134.1 ± 26.7	32.0
Total	114.0 ± 8.0	121.5 ± 18.2	7.0
Total number of entries			
Exp. 1	15.5 ± 0.9	9.4 ± 1.8	−39.0*
Exp. 2	16.6 ± 1.1	6.3 ± 1.2	−62.0‡
Total	16.6 ± 0.7	7.6 ± 1.1	−53.0‡

Number of entries and time spent in sec during the 5 min of the test are presented. In Experiment 1, 12 putrescine vs. saline pairs were studied and in Experiment 2, 16 pairs. Statistical significance of the difference between drug- and saline-injected littermates, $p<$, is indicated by: *0.002; †0.001; ‡0.0001.

black [−55.0%, $F(1,48)=42.0$, $p<0.0001$] compartments by approximately 50%, that is a 10–20% larger decrease than that observed with 400 mg/kg of putrescine. The total time spent in either white or black compartments was not significantly changed, which suggested that the average time per visit in each compartment was increased. Although the average time per visit showed the expected increase, this increase was not statistically significant. There was a good reproducibility between the two cyclohexylamine experiments.

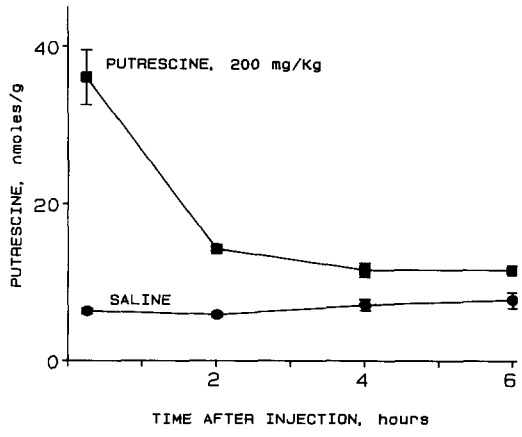

FIG. 1. The concentration of putrescine in brain cortex is shown 15 min, 2, 4, or 6 hours after injection of putrescine 200 mg/kg (squares) or saline (circles). Vertical bars are standard errors; where not visible, they are smaller than the size of the symbol. Each point is average of three cortices, therefore 24 rats were used. Saline and putrescine groups differed significantly, $F(1,16)=147$, $p\approx 0$. There was a significant difference with time, $F(3,16)=36$, $p\approx 0$, and a significant interaction between time and drug, $F(3,16)=41$, $p\approx 0$. The weight of the cortices in mg of saline and putrescine injected animals were respectively (mean ± SEM): 15 min, 836.1 ± 1.5, 848.9 ± 4.5; 2 hours, 882.6 ± 24.2, 833.6 ± 11.0; 4 hours 812.1 ± 4.6, 858.5 ± 16.9; 6 hours 825.5 ± 15.4, 859.3 ± 5.6.

Effect of the Pharmacological Treatments on the Concentration of Polyamines in Brain Cortex

The levels of polyamines in brain cortex were determined after injection of putrescine and cyclohexylamine. Fifteen min after injection of putrescine at 200 mg/kg the levels of putrescine in cortical homogenates increased six-fold; 6 hours later the levels were still higher than those in saline controls (Fig. 1). The levels of spermidine and spermine were not affected at any time. Putrescine 400 mg/kg had a similar effect to putrescine 200 mg/kg (Fig. 2). The main difference was that the rate of decrease of brain putrescine was slower at the higher dose. An unusually high dispersion of cortical putrescine concentration was found only in rats injected with 400 mg/kg of putrescine. After injection of 380 mg/kg of cyclohexylamine the levels of putrescine increased steadily for the first 4 hours (Fig. 3), while the concentrations of spermidine and spermine were not different from saline controls.

DISCUSSION

The test chosen for these studies, the Greek cross maze, measures the balance between the natural tendency of rodents to explore a new environment and the aversion to explore brightly

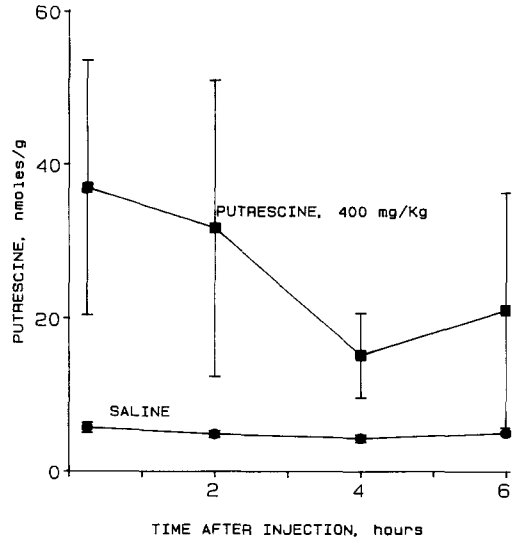

FIG. 2. Concentration of putrescine in brain cortices at the times indicated after IP injection of either putrescine 400 mg/kg or saline. A total of 24 rats was used. Each point is average of three cortices and the vertical bars are standard errors; where not visible bars are within area of symbol. Two-way ANOVA indicated a significant difference between saline and putrescine groups, $F(1,16)=7.96$, $p<0.012$. There were no significant changes with time, neither was there a significant interaction between factors drug and time. The weight of the cortices in mg of saline- and putrescine-injected animals were respectively (mean ± SEM): 15 min, 903.9 ± 27.0, 876.4 ± 32.5; 2 hours, 904.3 ± 13.1, 893.6 ± 11.2; 4 hours 881.3 ± 39.3, 882.8 ± 35.2; 6 hours 905.2 ± 52.6, 906.6 ± 35.1.

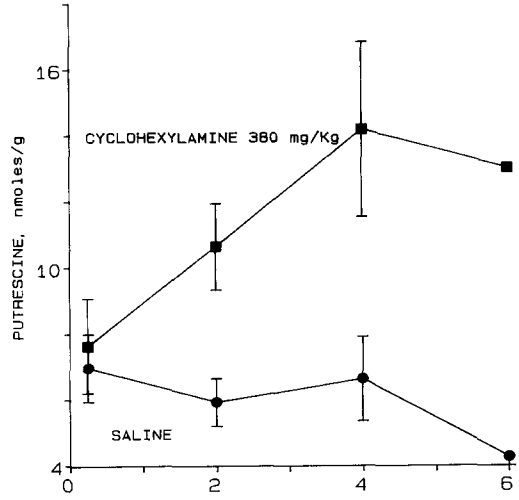

FIG. 3. Concentration of putrescine in brain cortex of rats 15 min, 2, 4 or 6 hours after injection of either 380 mg/kg of cyclohexylamine sulfate or saline. The number of cyclohexylamine versus saline pairs was 4 at 15 min after injection, 3 at 2 and 4 hours and one at 6 hours after injection. The total number of rats used was 22. The difference between saline and cyclohexylamine groups was statistically significant, $F(1,16)=6.29$, $p<0.023$. There was no significant effect of time, nor was there a significant interaction between time and drug factors. The weight of the cortices in mg of saline- and cyclohexylamine-injected animals were respectively (mean ± SEM): 15 min, 818.4 ± 18.3, 828.1 ± 32.7; 2 hours, 835.4 ± 27.8, 872.9 ± 23.8; 4 hours 877.6 ± 17.3, 877.7 ± 10.9; 6 hours 882.2, 880.9.

illuminated spaces. This balance depends on the emotional state of the animals; less emotional rats spent significantly more time in white compartments than more emotional ones (6).

Anxiogenic drugs decrease the exploration of bright areas, while anxiolytic drugs cause the opposite effect. A common way of measuring anxiety in rodents is the two-compartment paradigm, in which the animal can choose either to explore a smaller, dark compartment, or a larger, brightly illuminated one (3,5). The exploration is measured either as the number of crossings between bright and dark areas, the time spent in or the number of rearings in the bright area (10). This paradigm has been validated pharmacologically, behaviorally and physiologically (4).

Since the Greek cross maze and the Crawley and Goodwin (5) test confronts the animal essentially with the same choice of exploring either bright or dark compartments it is likely that both tests measure a similar behavioral state. This agrees with our preliminary results showing that diazepam (0.2 mg/kg and higher), an axiolytic drug, increased the number of entries into white compartments, while the β-carboline FG-7142 (20 mg/kg), an anxiogenic drug, had the opposite effect (results not shown).

Both putrescine and cyclohexylamine caused the animals to explore less. However, the lower dose of putrescine (200 mg/kg) decreased preferentially the entries and time spent in white compartments; the higher dose (400 mg/kg) and the cyclohexylamine, affected the entries into both white and black compartments without a significant effect on the time spent in them. We propose that at 200 mg/kg putrescine was anxiogenic, while at 400 mg/kg it exerted a general depressive or sedative activity. Although the possibility that drug-related malaise played some role in the present results cannot be ruled out, we observed no obvious signs of illness in our subjects, and others have concluded that putrescine is nontoxic in laboratory rodents at 200 and 400 mg/kg IP, even when administered repeatedly (9,17).

What is the relationship between the effect of these drugs on behavior and on brain polyamine levels? All three pharmacological treatments increased cortical putrescine concentration, without a measurable effect on spermidine or spermine concentrations. It could thus be postulated that the observed behavioral changes depend on the increase of brain putrescine levels. However, there was no correlation between the temporal course of changes in brain putrescine levels and behavior. With 200 mg/kg of putrescine there was an immediate almost six-fold increase in putrescine concentration followed by a sharp decrease. This decrease was not mirrored by a similar decrease in the behavioral effects of putrescine. A similar lack of temporal dependence between behavior and putrescine concentration was observed in the cyclohexylamine experiment. This could be explained by assuming that behavior is either influenced by a small pool of putrescine, which has limited equilibrium with the larger pool, or that behavior depends indirectly on the increase of brain putrescine concentration. In that respect it is worth noting that putrescine could affect anxiety by altering the metabolism of GABA: intraventricularly injected putrescine decreases GABA in chick brain by inhibiting glutamate decarboxylase, the enzyme that synthesizes GABA (13). The pivotal role of GABA and GABA receptor ligands in mood regulation is well documented (14).

ACKNOWLEDGEMENTS

We wish to thank Mr. Jose R. Cotto Aponte for the determination of polyamines, Mrs. Edna Rivera for her role in the behavioral observations and data analysis, and Dr. Richard Hann for proofreading the manuscript. This work was supported by RR08102 RCMI-NIH and RR08159 MBRS-NIH.

REFERENCES

1. Batchelor, K. W.; Smith, R. A.; Watson, N. S. Dicyclohexylamine is not an inhibitor of spermidine synthase. Biochem. J. 233:307–308; 1985.
2. Bennett, E. L.; Rosenzweig, M. R. Behavioral and biochemical methods to study brain responses to environment and experience. In: Lahue, R., ed. Methods in neurobiology, vol. 2. ed. New York: Plenum Press; 1981:101–141.
3. Blumstein, L. K.; Crawley, J. N. Further characterization of a simple automated exploratory model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 18:37–40; 1983.
4. Chopin, P.; Briley, M. Animal models of anxiety: the effect of compounds that modify 5-HT neurotransmission. Trends Pharmacol. Sci. 8:383–388; 1987.
5. Crawley, J.; Goodwin, F. K. Preliminary report of a simple animal behavioral model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 13:167–170; 1980.
6. DeNelsky, G. Y.; Denenberg, V. H. Infantile stimulation and adult exploratory behavior in the rat: effect of handling upon visual variation-seeking. Anim. Behav. 15:568–573; 1967.
7. Ferchmin, P. A.; Eterović, V. A. Role of polyamines in experience-dependent plasticity. Pharmacol. Biochem. Behav. 26:341–349; 1987.
8. Ferchmin, P. A.; Eterović, V. A.; Levin, L. E. Genetic learning deficiency does not hinder environment-dependent brain growth. Physiol. Behav. 24:45–50; 1980.
9. Genedani, S.; Piccinini, G.; Bertolini, A. Putrescine has analgesic activity, in rats. Life Sci. 34:2407–2412; 1984.
10. Kilfoil, T.; Michel, A.; Montgomery, D.; Whiting, R. L. Effects of anxiolytic drugs on exploratory activity in a simple model of anxiety in mice. Neuropharmacology 28:901–905; 1989.
11. Lynch, M. Mechanisms underlying induction and maintenance of long-term potentiation in the hippocampus. BioEssays 10:85–90; 1989.
12. McNaughton, B. L.; Barnes, C. A.; Rao, G.; Baldwin, J.; Rasmussen, M. Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information. J. Neurosci. 6:563–571; 1986.
13. Nistico, G.; Ientile, R.; Rotiroti, D.; Di Giorgio, R. M. GABA depletion and behavioral changes produced by intraventricular putrescine in chicks. Biochem. Pharmacol. 29:954–957; 1980.
14. Polc, P. Electrophysiology of benzodiazepine receptor ligands: multiple mechanisms and sites of action. Prog. Neurobiol. 31:349–424; 1988.
15. Qi, D. F.; Schatzman, R. C.; Mazzei, G. J.; Turner, R. S.; Raynor, R. L.; Liao, S.; Kuo, J. F. Polyamines inhibit phospholipid-sensitive and calmodulin-sensitive Ca^{2+}-dependent protein kinases. Biochem. J. 213:281–288; 1983.
16. Ransom, R. W.; Stec, N. L. Cooperative modulation of [^{3}H]MK-801 binding to the N-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. J. Neurochem. 51:830–836; 1988.
17. Rosenthal, S. M.; Fisher, E. R.; Stohlman, E. F. Nephrotoxic action of spermine. Proc. Soc. Exp. Biol. 80:432–434; 1952.
18. Smith, C. D.; Snyderman, R. Modulation of inositol phospholipid metabolism by polyamines. Biochem. J. 256:125–130; 1988.