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Acute Intracerebroventricular Injections of the Mast Cell Degranulator Compound 48/80 and Behavior in Rats

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LEWIS, S. J., M. J. QUINN, M. R. FENNESSY AND B. JARROTT. *Acute intracerebroventricular injections of the mast cell degranulator Compound 48/80 and behavior in rats.* PHARMACOL BIOCHEM BEHAV **33**(1) 75–79, 1989.—The intracerebroventricular (ICV) injection of the mast cell degranulator Compound 48/80 (2.5–20.0 μg/kg) produced a marked behavioral syndrome in normotensive rats. The behaviors included head and body shakes, paw tremor, excessive grooming, unusual posture and gait, mild diarrhoea, piloerection, extreme agitation and irritability to touch, and a later phase of sedation. The highest doses (15 and 20 μg/kg) also produced catalepsy and episodes of "barrel rolling" (continuous rolling of 1–8 turns around the longitudinal axis). These behaviors were observed for approximately 15–30 min although the sedation and catalepsy were maintained for 90–120 min. A second ICV injection of the 10 μg/kg dose of Compound 48/80 given 2 hr after an initial injection of this dose, produced a much reduced response and the numbers of head and body shakes, and episodes of paw tremor and grooming were between 20–30% of those produced by the first injection. The reduced effect of the second injection indicates that the behavioral effects of Compound 48/80 may arise from the acute degranulation of mast cells rather than direct effects on neuronal populations or the cerebral vasculature.

Intracerebroventricular Compound 48/80 Brain mast cells Rats Head and body shakes Paw tremor and other behaviors

IT has been established that mast cells exist in the mammalian central nervous system (8, 9, 13). In rats, the brain regions which contain significant numbers of mast cells include the cortex, the dorsolateral aspects of the hypothalamus, the median raphe and the grisea of the brain stem (8, 9, 13). These cells are strictly localized around blood vessels (arterioles and venules) except in the leptomeninges over the brain surface and in the choroid fissures where a nonvascular distribution is more common (13). Although the physiological functions of mast cells in the brain have not been established precisely, roles in defensive inflammatory responses and tissue repair, myelination and the metabolism of sulphate and lipids have been proposed [see (8, 9,13)]. In addition, it has been suggested that the biogenic amines histamine, serotonin and dopamine, which are stored and released by brain mast cells, may serve physiological roles as neuromodulators or as regulators of cerebrovascular haemodynamics [see (9,13)]. At present, little is known about the role of mast cells in behavioral control and whether or not disturbances in mast cell function will result in behavioral disorders. The aim of the present study was to examine the acute effects of intracerebroventricular injections of the potent mast cell degranulator Compound 48/80, a condensation product of N-methyl-p-methoxyphenethylamine and formaldehyde (23) on the behavior of normotensive unanaesthetized Wistar rats.

METHOD

Animals

Female Wistar rats weighing between 210–242 g were used. From five days prior to, and during the experiments, the rats were kept in individual cages in a room with a 12-hr light (0800–2000 hr) 12-hr dark cycle and an ambient temperature of 21 ± 1°C. Food and water were available ad lib.

Surgery

The rats were anaesthetized with a methohexitone (16.7 mg/

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ml)/amylobarbitone sodium (30 mg/ml) mixture (given 1 ml/kg body weight) and then placed in a stereotaxic apparatus. The left lateral ventricle of each rat were surgically implanted with a permanent indwelling polyethylene (PE-10) cannula using the coordinates 2 mm lateral to the sagittal suture and 1 mm caudal to the coronal suture (22). The rats were allowed 4 days to recover before use.

Experimental Procedure

At the time of the experiment (1100–1400 hr) the free end of the ventricular cannula was fitted to a length of PE-10 tubing which was connected to an Agla micrometer all glass syringe. The Compound 48/80 (2.5–20.0 μg/kg dissolved in 0.9% w/v saline) was injected in a 10 μl volume. This was immediately followed by 10 μl of saline to ensure complete administration of the drug. Control rats received 20 μl of saline. The 20 μl (total) volumes were delivered over a 30-sec period. The behaviors of the rats (see Table 1) were assessed quantitatively and/or qualitatively as described previously (17). In brief, 'head shakes' represent paroxysmal shakes of the head only, whereas 'body shakes' consist of paroxysmal shakes of the head and torso (reminiscent of a dog shaking off water). 'Paw tremor' consists of rapid, apparently uncontrolled, bursts of movement of the front paws, whereas 'backward kicks' represent more deliberate (but rapid) arching backwards of the hindlegs. 'Barrel rolling' represents episodes of continuous rolling (1–8 turns) around the longitudinal axis. 'Excessive grooming' represents episodes of grooming of the head and torso which are either sustained for longer than normal or are repeated at a much higher frequency. The same observational procedure allowed for the evaluation of irritability, sedation and catalepsy. At the time of the experiments (1100–1400), the rats were generally lying quietly in their test cages (after a period of acclimatization, usually 15–30 min). Normally, rats do not object to being handled. If there is an obviously abnormal reaction to handling, e.g., biting or vocalization, this is referred to as 'irritability to touch.' If, upon handling, the rats show less reaction than normal, i.e., little attempt to escape or investigation of the environment outside their cages. This is referred to as 'sedation' if the rats are shown not to be cataleptic. 'Catalepsy' was tested by placing the rats on the lip of the open cages. Cataleptic rats remain motionless and cling to the lip of the cage and will not drop as normal animals do. In this study the behaviors of the control rats and those receiving Compound 48/80 were monitored for 15 min prior to, and until 120 min after injection.

Drugs

Compound 48/80 (condensation product of N-methyl-p-methoxyphenethylamine and formaldehyde) was obtained from Sigma Chemical Corporation, St. Louis, MO.

Statistics

The data were analysed by Friedman's two-way nonparametric analysis of variance (ANOVA) for repeated measures (between times comparisons) data followed by the critical range test for examination of differences between individual means, or, the Kruskall-Wallis one-way nonparametric ANOVA, for nonrepeated measures data (i.e., between dose comparisons with different animals in each group) followed by the critical range test (5) or where appropriate, ANOVA and Student's modified *t*-test with the Bonferroni adjustment for multiple comparisons (29). A value of $p<0.05$ is considered to be significant.

RESULTS

The intracerebroventricular injection of Compound 48/80 (2.5–20 μg/kg) produced a marked, dose-dependent behavioral syndrome in rats (Tables 1 and 2). This syndrome consisted of head and body shakes, excessive grooming, paw tremor, biting, gnawing and licking movements, piloerection, agitation and irritability to touch, mild diarrhoea, occasional backward kicking, a latter phase of sedation, and following the higher doses (15 and 20 μg/kg), catalepsy and "barrel rolling" (continuous rolling around the longitudinal axis). The majority of these behaviors began within 1–2 min of administration, were most predominant between 5–15 min and had subsided by 30 min (Table 2). The sedation and catalepsy following the 2.5–10 μg/kg doses were established by 10–15 min and were sustained for approximately 90–120 min. These latter effects were more pronounced and were established earlier (between 5–10 min) following the administration of the 15 and 20 μg/kg doses. For all doses of Compound 48/80 (see Table 2), the behaviors were most evident over the 0–15 min postinjection period. All of these values were greater than those over the 15–30 min and 30–120 min periods ($p<0.05$ for all comparisons) and those occurring between 15–30 min were greater than those occurring between 30 and 120 min ($p<0.05$ for all comparisons). In terms of dose-response, the order of potency in producing the behaviors was for the 0–15 min postinjection period (2.5 = 5<10<15 = 20 μg/kg; significance of between dose differences determined by Kruskull-Wallis plus critical range tests; $p<0.05$), the 15–30 min period (2.5<5 = 10 = 15 = 20 μg/kg) and for the 30–120 min period (2.5 = 5 = 10<15 = 20 μg/kg). The 5 and 10 μg/kg doses of Compound 48/80 produced the maximal numbers of head shakes (Fig. 1), whereas the 5 μg/kg dose produced the greatest number of body shakes ($p<0.05$ for comparisons between

TABLE 1

THE EFFECTS OF INTRACEREBROVENTRICULAR (ICV) INJECTIONS OF EITHER SALINE (0.9% w/v) OR DIFFERENT DOSES OF COMPOUND 48/80 (μg/kg) ON THE GENERAL BEHAVIOR OF RATS

	Compound 48/80 (μg/kg)					
Behavior	Saline	2.5	5.0	10.0	15.0	20.0
Head Shakes	0/+	++	+++	+++	++	+
Body Shakes	0	++	+++	++	++	+
Sedation	0	0	+	++	+++	+++
Catalepsy	0	0	0	+	++	+++
Unusual Posture/Gait*	0	0	+	++	++	+++
Diarrhoea	0	0	+	+	+	+
Backward Kicks	0/+	+	++	++	+	+
Paw Tremor	0/+	+	+++	+++	++	+
Piloerection	0	+	++	++	++	++
"Barrel Rolling"†	0	0	0	0	+	++
Irritability to Touch	0	++	+++	+++	++	++
Loss of Righting Reflex	0	0	0	0	0	0/+
Excessive Grooming	0	0	++	++	++	+
Gnawing/Biting Movements	0	+	++	++	++	++

*Includes splayed front and/or back legs.

†Continuous rolling (1–8 turns) around longitudinal axis.

The appearance and extent of each behavior was assessed subjectively as follows: 0, no change; +, slight change; ++, moderate change; and +++ large change. The behaviors of the rats during the 15 min period immediately prior to injection was compared to those which occurred 0–15 min immediately following injection. Each dose was given to a separate group of rats (n = 5 or 6 rats per group). Each value represents the average score for each group.

TABLE 2

THE TIME-COURSE OF EFFECTS OF EITHER SALINE (0.9% w/v) OR DIFFERENT DOSES OF COMPOUND 48/80 (2.5–20 μg/kg) ON THE GENERAL BEHAVIOR OF RATS

		Mean Total Test Score*†			
		Time Before or After Injection (min)			
Dose	n	−15 to 0	0 to 15	15 to 30	30 to 120
0 (saline)	5	0.4	0.8	0.6	0.6
2.5	4	0.4	4.5‡	2.0‡	0.5
5.0	5	0.6	5.4‡	2.8‡	0.8
10.0	5	0.6	6.8‡	3.2‡	1.0
15.0	5	0.4	9.0‡	3.4‡	2.0‡
20.0	5	0.6	8.0‡	3.8‡	1.8‡

*Over each time interval either before or after injection, the presence or absence of 10 behaviors were noted and scored (0 = absent, 1 = present; maximal mean total score = 10). The 10 behaviors are: Head shakes, body shakes, sedation, unusual posture/gait, diarrhoea, backward kicks, paw tremor, piloerection, "barrell rolling" and excessive grooming. Although the test scores do not reflect the intensity of any individual behavior, it should be noted that the frequency of head shakes following 10 μg/kg Compound 48/80 were as high as 6 per min (see Fig. 1).

†See text for between dose comparisons.

‡Significant from preinjection values at $p<0.05$–0.0001, by Friedman's two-way ANOVA plus critical range test (see the Method section).

all means using ANOVA followed by Student's modified *t*-test with the Bonferroni correction for multiple comparisons). The 5 and 10 μg/kg doses also produced the maximal numbers of paw tremors and grooming episodes and also irritability. As such, the enhanced sedative and cataleptic effects of the two highest doses may have prevented the full expression of these other behaviors. A second injection of the 10 μg/kg dose of Compound 48/80 given 2 hr after the first injection produced a much reduced behavioral response (Table 3). The numbers of head and body shakes, paw tremors and grooming episodes were between 20–30% of those

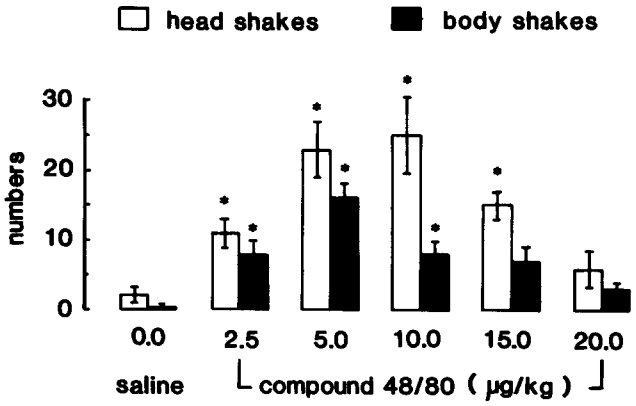

FIG. 1. The numbers of head and body shakes (expressed as mean ± s.e.m.) observed over the 15 min period immediately following the ICV injection of either saline (0.9% w/v) or Compound 48/80 (2.5–20 μg/kg) to unrestrained rats. Each dose of Compound 48/80 was given to separate groups of rats (n = 5 animals per group) *$p<0.05$ compared to that of saline as determined by ANOVA followed by Student's modified *t*-test with the Bonferroni correction for multiple comparisons.

TABLE 3

A COMPARISON OF THE BEHAVIORAL EFFECTS OF A SECOND ICV INJECTION OF COMPOUND 48/80 (10 μg/kg) GIVEN 2 HR AFTER THE FIRST SUCH INJECTION

	1st Injection		2nd Injection	
Behavior	Saline	Compound 48/80	Saline	Compound 48/80
Head Shakes	2 ± 1	24 ± 3*	3 ± 1	8 ± 2†
Body Shakes	1 ± 1	10 ± 2*	2 ± 1	3 ± 2†
Grooming Episodes	3 ± 1	28 ± 4*	3 ± 1	2 ± 1†
Paw Tremor	2 ± 1	16 ± 3*	2 ± 1	3 ± 2†

*$p<0.05$, 1st injection of Compound 48/80 versus saline, ANOVA followed by Student's modified *t*-test with the Bonferroni correction for multiple comparisons.

†$p<0.05$, 2nd injection versus 1st injection, statistics as above.

The behaviors were recorded for 15 min after each injection. Each value represents the mean ± S.E.M. (n = 6 rats per group).

produced by the first injection. In addition, the second injection did not produce backward kicking, diarrhoea or catalepsy although the rats did display a relatively mild sedation.

DISCUSSION

The present study demonstrates that the acute ICV injection of Compound 48/80 produces a marked behavioral syndrome in rats. The most marked features of this syndrome included head and body shakes, paw tremor, extreme agitation and irritability to touch, and in higher doses catalepsy and "barrel rolling." Although Compound 48/80 is a potent degranulator of central (13) and peripheral (23) mast cells, it also has, in higher concentrations, other pharmacological actions which include the accumulation of cyclic adenosine 3′,5′-monophosphate (cAMP) in the brain (20) and superior cervical ganglion (19), the blockade of cholinergic neurotransmission in the cat superior cervical ganglion (6), blockade of rabbit-neuromuscular junction (26), inhibition of the negative chronotropic effect of vagal nerve stimulation in guinea pig atria (16) and the stimulation of small granule containing cells in the rat superior cervical ganglion (2). In addition, it has been suggested that Compound 48/80 may act as a selective and competitive antagonist at opiate receptors located on cholinergic nerve terminals in the guinea pig ileum (15). These observations suggest that some of the behavioral effects of ICV Compound 48/80 may arise from actions other than mast cell degranulation, and in particular, the modulation of cholinergic and opioid neurotransmission and the accumulation of cAMP. Nevertheless, the much reduced behavioral effects of a second injection of Compound 48/80 (10 μg/kg given 2 hr after the initial injection) supports the suggestion that the behaviors may indeed arise from the acute degranulation of mast cells. Since the restoration of mast cell contents following degranulation takes between 8–24 hr (13,24), the reduced effect of the second injection of Compound 48/80 is consistent with the first such injection degranulating a significant proportion of the mast cells accessible via the ICV route. It is unlikely that a Compound 48/80-induced blockade of central opiate receptors is primarily responsible for the behavioral syndrome as the selective opiate antagonists naloxone and naltrexone have little overt effects on behavior in rats or other species (3,12) although very high doses (above 10 mg/kg) do produce unusual behaviors including retching, vomiting and profuse salivation and frothing at the mouth in squirrel monkeys (4). To our

knowledge the administration of high doses of naloxone or naltrexone in rats have not been reported to produce adverse behavioral effects.

Unfortunately, there is no data available on the extent and rate of diffusion of Compound 48/80 throughout the brain after ICV injection. However, the relatively rapid onset of most of the behaviors (i.e., within 1–2 min of injection) would suggest that mast cell containing structures in closer proximity to the ventricles, e.g., the dorsolateral hypothalamus, may be involved. Moreover, the occurrence of 'barrel-rolling' suggests that the predominant mast cell degranulation may occur initially in tissue located ipsilaterally to the injected Compound 48/80. However, it is likely that the majority of the effects of Compound 48/80 involve brain structures both contralateral and ipsilateral to the ventricle of injection. In addition, since significant numbers of mast cells have also been identified in the cortex, median raphe and the brainstem (8, 9, 13) these structures are likely to be involved in the actions of Compound 48/80.

Although brain mast cells store and release the biogenic amines, histamine and serotonin, they also contain large numbers of other compounds including heparin, phospholipids and a wide variety of hydrolytic enzymes including aminopeptidases, lipases and peroxidases (13). Consequently, the behavioral response following the degranulation of mast cells by ICV Compound 48/80 may involve any number of these compounds. However, the injection of histamine into discrete brain sites produces a variety of effects including head and body shakes, grooming, irritability to touch and catalepsy (11), which are qualitatively similar to those observed following ICV Compound 48/80. Moreover, central serotonergic mechanisms have also been implicated in the mediation of body shaking behavior (1). These observations suggest that a Compound 48/80-induced release of histamine and/or serotonin from mast cells may be involved in producing at least some of the observed behaviors. The manner by which the released histamine and serotonin elicit these behaviors may involve several mechanisms. It is possible that these amines directly interact with the various neuronal pathways involved in behavioral control. However, the close proximity of mast cells to the cerebral vasculature (8, 9, 13) suggest that an acute degranulation of these cells may lead to significant disturbances in cerebral blood flow which in turn may underlie the behavioral disturbances. The precise mechanisms of action remain to be determined.

An interesting finding of this study is that many of the behaviors produced by ICV Compound 48/80 and in particular the head and body shakes, paw tremor, piloerection and excessive grooming also occur in the morphine (17), clonidine (14) and delta-^{9}-tetrahydrocannabinol (27,28) abstinence syndromes in rats. In addition, central histaminergic (18, 21, 27) and serotonergic (17, 18, 28) mechanisms (presumed to be neuronal) have been implicated in all of these syndromes. The present study suggests the possibility that an increase in release in histamine and serotonin from mast cells may also be involved in the expression of the behavioral aspects of abstinence syndromes in general. It has been reported that peripheral mast cells contain beta-endorphin (7). Although it is not known if central mast cells contain this endogenous opiate, a Compound 48/80-induced release of beta-endorphin from brain mast cells would be consistent with many of the observed behaviors and in particular the excessive grooming, body shakes and catalepsy (10,25).

In summary, the present study demonstrates that the acute disruption of brain mast cells produces behavioral phenomena similar to those of abstinence syndromes. These findings suggest that an alteration in mast cell function may be involved in the aetiology of behavioral disturbances in general.

REFERENCES

1. Bedard, P.; Pycock, C. J. 'Wet-dog' shake behavior in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacology 16:663–670; 1977.
2. Behrendt, H.; Lindl, T.; Cramer, H. *In vitro* effects of histamine liberator Compound 48/80 on rat superior cervical ganglia with special regard to the small granule containing cells. Cell Tissue Res. 166:70–81; 1976.
3. Byrd, L. D. Effects of morphine alone and in combination with naloxone or d-amphetamine on shock-maintained behaviour in the squirrel monkey. Psychopharmacology (Berlin) 49:255–234; 1976.
4. Byrd, L. D. Cardiovascular effects of naloxone, naltrexone and morphine in the squirrel monkey. Life Sci. 32:391–398; 1983.
5. Colquhoun, D. Lectures on biostatistics. Oxford: Clarendon Press; 1971:191–213.
6. Damjanovich, S. O.; Feher, O.; Bokri, E. The effects of 48/80 on the transmission of impulses in a sympathetic ganglion. Arch. Int. Pharmacodyn. 141:490–499; 1963.
7. Di Augustine, R. P.; Lazarus, J. H.; Jahnke, G.; Erisman, M. N.; Linnoila, R. I. Corticotropin/beta-endorphin immunoreactivity in rat mast cells. Peptide or protease? Life Sci. 2663–2668; 1980.
8. Dropp, J. J. Mast cells in the central nervous system of several rodents. Anat. Rec. 174:227–238; 1972.
9. Edvinsson, L.; Cervos-Navarro, J.; Larsson, L. I.; Owman, Ch.; Ronnberg, A. L. Regional distribution of mast cells containing histamine, dopamine or 5-hydroxytryptamine in mammalian brain. Neurology 27:878–883; 1977.
10. Gispen, W. H.; Wregant, V. M.; Bradbury, A. F.; Hume, E. C.; Smyth, D. G.; Snell, C. R.; deWeid, D. Induction of excessive grooming in the rat by fragments of lipotropin. Nature 264:794–795; 1976.
11. Glick, S. D.; Crane, L. A. Opiate-like and abstinence-like effects of intracerebral histamine administration in rats. Nature 273:547–549; 1978.
12. Holtzman, S. G. Effects of morphine and narcotic antagonists on avoidance behavior of the squirrel monkey. J. Pharmacol. Exp. Ther. 196:145–155; 1976.
13. Ibrahim, M. Z. M. The mast cells of the mammalian central nervous system. Part 1. Morphology, distribution and histochemistry. J. Neurol. Sci. 21:431–478; 1974.
14. Jarrott, B.; Lewis, S. J.; Conway, E. L.; Summers, R. J.; Louis, W. J. The involvement of central alpha adrenoceptors in the antihypertensive actions of methyldopa and clonidine in the rat. Clin. Exp. Hypertens. 6:387–400; 1984.
15. Kamikawa, Y.; Shimo, Y. Antagonist effect of Compound 48/80 on the inhibitory actions of morphine and methionine-enkephalin on electrically-induced contractions of the guinea-pig ileum. Br. J. Pharmacol. 64:511–518; 1978.
16. Kasuya, Y.; Kamikawa, Y. Studies on the cardiac anaphylaxis in the guinea-pig. 1. changes in the sensitivity to some drugs induced by anaphylactic desensitization. Jpn. J. Pharmacol. 23(Suppl):144; 1973.
17. Laska, F. J.; Fennessy, M. R. Physical dependence in the rat induced by slow release morphine: dose response, time course and brain biogenic amines. Clin. Exp. Pharmacol. Physiol. 3:587–598; 1976.
18. Lewis, S. J.; Fennessy, M. R.; Taylor, D. A. Central aminergic mechanisms associated with the clonidine withdrawal syndrome. Clin. Exp. Pharmacol. 4:449–455; 1981.
19. Lindl, T.; Behrendt, H.; Heinl-Sawaya, M. C. B.; Tenfel, E.; Cramer, H. Effects of Compound 48/80 on mast cells, histamine and cyclic AMP in isolated superior cervical ganglia. Naunyn Schmiedebergs Arch. Pharmacol. 286:283–296; 1974.
20. Lindl, T.; Heinl-Sawaya, M. C. B.; Cramer, H. Effect of Compound 48/80, a histamine releasing agent, on accumulation and release of cyclic AMP in various regions of rat brain *in vitro*. Res. Commun. Chem. Pathol. Pharmacol. 13:65–74; 1976.
21. Mazurkiewizc-Kwilecki, I.; Henwood, R. W. Alterations in brain endogenous histamine levels in rats after chronic morphine treatment and morphine withdrawal. Agents Actions 6:402–408; 1976.
22. Noble, E. P.; Wurtmann, R. J.; Axelrod, J. A simple and rapid

method for injecting ^{3}H-norepinephrine into the lateral ventricle of the rat brain. Life Sci. 6:281–291; 1967.
23. Riley, J. F.; West, G. B. Tissue mast cells. Studies with a histamine liberator of low toxicity (Compound 48/80). J. Pathol. Bacteriol. 69:269–282; 1955.
24. Schwartz, J-C. Histamine as a transmitter in brain. Life Sci. 17: 503–518; 1975.
25. Segal, D. A.; Brown, R. G.; Arnsten, H.; Cerrington, D. C. Behavioral effects of beta-endorphin. In: Van Ree, J. M.; Terenius, L., eds. Characteristics and function of opioids. Amsterdam: North Holland Press; 1979:337–386.
26. Somjen, G.; Uyldert, E. The effect of Compound 48/80 on mammalian skeletal muscle. Br. J. Pharmacol. Chemother. 10:413–417; 1955.
27. Verberne, A. J. M.; Fennessy, M. R.; Lewis, S. J.; Taylor, D. A. Involvement of brain histamine in delta-^{9}-tetrahydrocannabinol tolerance and dependence. Pharmacol. Biochem. Behav. 23:153–159; 1985.
28. Verberne, A. J. M.; Taylor, D. A.; Fennessy, M. R. Withdrawal-like behavior induced by inhibitors of biogenic amine reuptake in rats treated chronically with Δ^{9}-tetrahydrocannabinol. Psychopharmacology (Berlin) 68:261–267; 1980.
29. Wallenstein, S.; Zucker, C. I.; Fleiss, J. L. Some statistical methods useful in circulation research. Circ. Res. 47:1–9; 1980.