Pharmacology Biochemistry & Behavior, Vol. 36, pp. 317–320. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Effects of Neurotropic Agents With a Selectivity for Alpha-Adrenoceptors on Nitrile-Induced Dyskinetic Syndrome in Mice

HIDEJI TANII, MASAO HAYASHI AND KAZUO HASHIMOTO

Department of Hygiene, School of Medicine, Kanazawa University
13-1, Takara-machi, Kanazawa 920, Japan

Received 12 June 1989

TANII, H., M. HAYASHI AND K. HASHIMOTO. *Effects of neurotropic agents with a selectivity for alpha-adrenoceptors on nitrile-induced dyskinetic syndrome in mice.* PHARMACOL BIOCHEM BEHAV **36**(2) 317–320, 1990.—Allylnitrile, crotononitrile and 2-pentenenitrile induce this behavioral syndrome, which includes persistent random circling, head twitching and backward running. The effects of neurotropic agents, with a selectivity for the $alpha_1$ or $alpha_2$ adrenoceptor, were examined on the nitrile-induced behavioral abnormalities. The $alpha_1$ agonist phenylephrine, the $alpha_2$ agonist clonidine and the $alpha_1$ antagonist prazosin, inhibited the behavioral syndrome induced by each nitrile, while the $alpha_2$ antagonist yohimbine had no effect. These results suggest that the noradrenergic system may be involved in the manifestation of the syndrome. Furthermore, a tonic noradrenergic influence on subcortical systems may be required for the syndrome to occur.

Allylnitrile Crotononitrile 2-Pentenenitrile Clonidine Phenylephrine Yohimbine Prazosin
Adrenoceptor Persistent behavioral abnormalities

WE have recently shown that a single oral dose of allylnitrile ($CH_2=CHCH_2CN$) in mice pretreated with CCl_4 induces behavioral abnormalities such as circling, hyperactivity, head twitching and occasional backward running, lasting throughout a 4-month observation period (27). In our current study, the dyskinetic syndrome was found to last throughout the lifespan of the animals (unpublished observation). These findings were also true in the case of crotononitrile ($CH_3CH=CHCN$) and 2-pentenenitrile ($CH_3CH_2CH=CHCN$) (28). These persistent abnormalities were similar to those produced by 3,3′-iminodipropionitrile (IDPN) (1, 8, 21) and phencyclidine (12,24).

So far the behavioral syndrome induced by serotonin (5-HT) or IDPN has been used as a model for human basal ganglia disorders (3–6, 13, 14, 16, 22), and the relation of neurotransmitter interactions to the syndrome has also been studied. The IDPN-induced syndrome has been shown to be inhibited by many neurotropic agents which act on 5-HT, dopamine, or norepinephrine receptors (5, 7, 22). The syndrome induced by allylnitrile, crotononitrile or 2-pentenenitrile is inhibited by various drugs which act on different receptors, including serotonergic and dopaminergic antagonists (27,28). It remained unknown whether or not other neurotransmitters were also involved in the manifestation of these behavioral abnormalities.

The present study describes the effects of these drugs with selelctive actions on alpha-adrenergic receptors, in connection with the allylnitrile-, crotononitrile- or 2-pentenenitrile-induced syndromes, since $alpha_1$-adrenergic antagonists as well as $alpha_2$-agonist clonidine, have been shown to inhibit the syndrome by 5-HT and IDPN (5,13). Studies on the effects of the drugs were done two months after dosing with each nitrile, because the mice showed various dyskinetic behaviors more than one month after dosing with the nitriles used in this study (27,28).

METHOD

Allylnitrile, crotononitrile, 2-pentenenitrile, clonidine hydrochloride, and prazosin hydrochloride were obtained from Tokyo Kasei Co. (Tokyo); yohimbine hydrochloride from Wako Pure Chemical Industries (Osaka); and L-phenylephrine hydrochloride from Sigma Chemical Co. (St. Louis, MO). All chemicals were reagent grade.

Male mice of ddY strain weighing about 25 g at the beginning of the experiment were maintained on a 12-hr light, 12-hr dark cycle with free access to laboratory chow and water. Based on our findings previously reported (25), the animals were pretreated intraperitoneally with 0.16 ml of a 20% CCl_4 solution in olive oil per 25 g body weight, in order to inhibit the microsomal oxidizing enzymes (25,26). Twenty-four hours later, these animals were dosed orally with either allylnitrile (150 mg/kg), crotononitrile (283 mg/kg) or 2-pentenenitrile (162 mg/kg), each test compound being dissolved in olive oil, in a volume of 0.1 ml per 25 g. The dose levels employed were based on our previous studies (27,28)

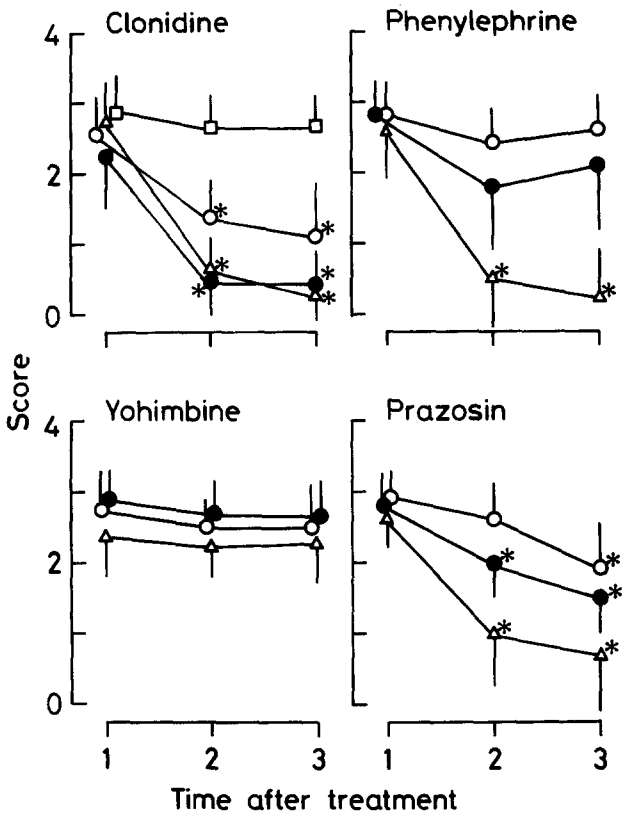

FIG. 1. Effects of clonidine, phenylephrine, yohimbine and prazosin on allylnitrile-induced dyskinetic syndrome. Clonidine: 0 (□), 0.05 (○), 0.1 (●), and 0.2 (△) mg/kg. Phenylephrine: 2.0 (○), 4.0 (●), and 8.0 (△) mg/kg. Yohimbine: 2.0 (○), 4.0 (●), and 8.0 (△) mg/kg. Prazosin: 1.0 (○), 2.0 (●), and 4.0 (△) mg/kg. Prior to the drug administration, mice were observed for 5 min for baseline recording (the number 1 in the horizontal). The animals were then dosed with each drug or vehicle, and the observation and scoring were made over periods of 15 to 20 (the number 2) and 30 to 35 min (the number 3). Each value represents the mean ± S.D. of eight animals. *$p<0.05$ vs. control value (baseline) by Dunnett's multiple comparison procedure.

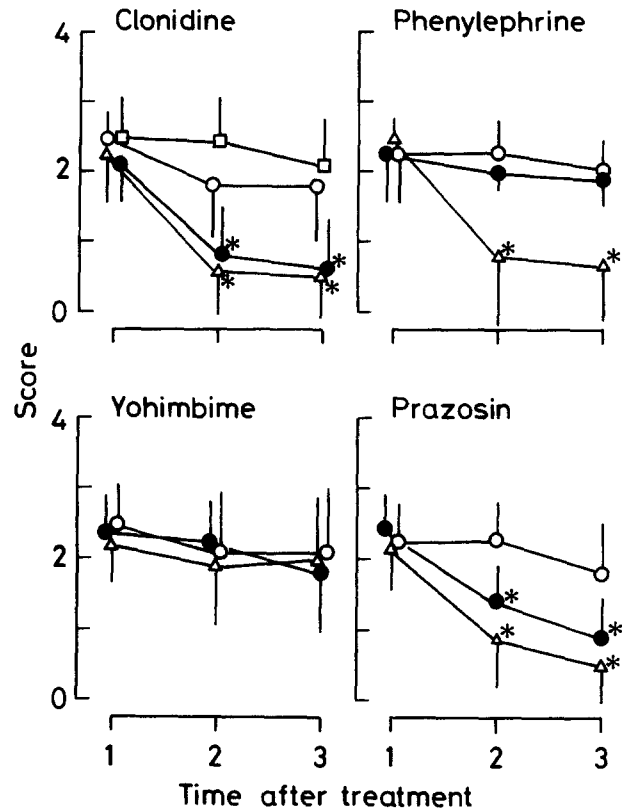

FIG. 2. Effects of clonidine, phenylephrine, yohimbine and prazosin on crotononitrile-induced dyskinetic syndrome. Clonidine: 0 (□), 0.05 (○), 0.1 (●), and 0.2 (△) mg/kg. Phenylephrine: 2.0 (○), 4.0 (●), and 8.0 (△) mg/kg. Yohimbine: 2.0 (○), 4.0 (●), and 8.0 (△) mg/kg. Prazosin: 1.0 (○), 2.0 (●), and 4.0 (△) mg/kg. The numbers in the horizontal represent the same as Fig. 1. Each value represents the mean ± S.D. of eight animals. *$p<0.05$ vs. control value (baseline) by Dunnett's multiple comparison procedure.

in which each nitrile induced the same persistent dyskinetic syndrome in mice. Two months after dosing the dyskinetic behaviors were assessed using a dyskinesia scale (5): score 0 represented absence of behavioral abnormalities; score 1 included random circling; score 2, vertical dyskinetic head and neck movements (retrocollis); score 3, circling and retrocollic movements; score 4, lateral head weavings or shakings. Observation of behavior was done between 0900 and 1500 by one of the authors who had no knowledge of the treatments.

The drugs employed were clonidine (0.05, 0.1 and 0.2 mg/kg), phenylephrine (2.0, 4.0 and 8.0 mg/kg), yohimbine (2.0, 4.0 and 8.0 mg/kg) and prazosin (1.0, 2.0 and 4.0 mg/kg). The drugs were dissolved in saline, except for prazosin which was dissolved in saline with heating. Drug solutions were prepared just prior to use and administered intraperitoneally in a volume of 0.1 ml/25 g. Control animals received the same volume of vehicle. On the day of testing, mice were placed individually in a cage and left for 20 min, after which each animal was observed for 5 min for baseline behaviors. They were then injected intraperitoneally with each drug or vehicle, and the observation and scoring were made over periods of 15 to 20 and 30 to 35 min, because the neurotropic agents used in this study have been shown to exhibit the effects within 35 minutes as previously reported (5).

The dyskinetic behavior was expressed as the mean ± S.D. score of eight mice and was statistically analyzed using the analysis of variance, followed by Dunnett's multiple comparisons with controls (11).

RESULTS

Allylnitrile-Induced Dyskinesia

The effects of the drugs on the allylnitrile-induced dyskinetic syndrome are shown in Fig. 1. The alpha$_2$-adrenoceptor agonist clonidine reduced the behavioral abnormalities induced by allylnitrile, $F(3,92) = 15.83$, $p<0.01$. These effects were seen at dose levels of 0.05, 0.1 and 0.2 mg/kg over an observation period of 35 min. The alpha$_1$-adrenoceptor agonist phenylephrine inhibited the dyskinesia at a dose level of 8.0 mg/kg over a period of 35 min, but not at dose levels of 2.0 and 4.0 mg/kg, $F(3,92) = 15.77$, $p<0.01$. The alpha$_2$-adrenoceptor antagonist yohimbine (2.0–8.0 mg/kg) had no effect on the dyskinetic score. Following acute administration of the alpha$_1$-adrenoceptor antagonist prazosin, there was an attenuation in the behavioral abnormalities, $F(3,92) = 8.98$, $p<0.01$. Prazosin caused a significant decrease in the dyskinesia score at a dose level of 1.0 mg/kg 30 to 35 min after

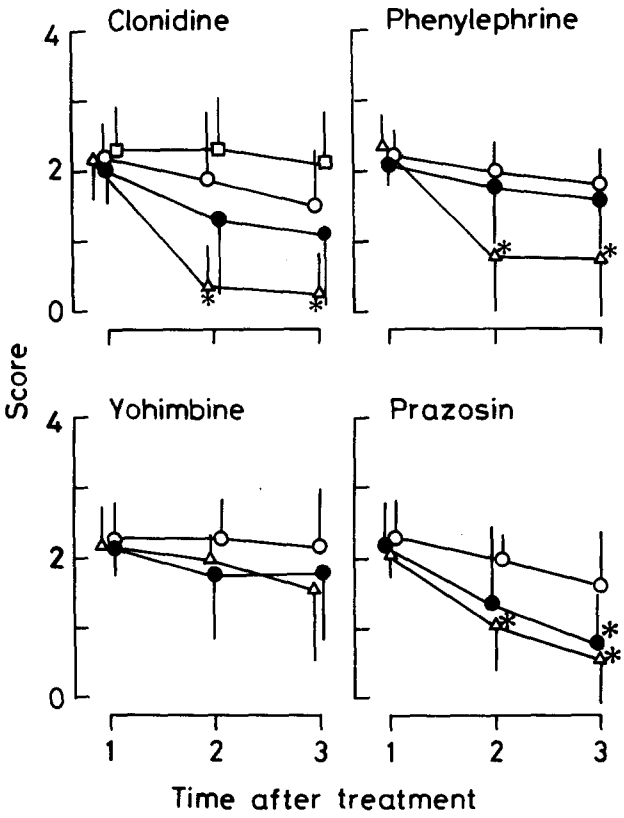

FIG. 3. Effects of clonidine, phenylephrine, yohimbine and prazosin on 2-pentenenitrile-induced dyskinetic syndrome. Clonidine: 0 (□), 0.05 (○), 0.1 (●), and 0.2 (△) mg/kg. Phenylephrine: 2.0 (○), 4.0 (●), and 8.0 (△) mg/kg. Yohimbine: 2.0 (○), 4.0 (●), and 8.0 (△) mg/kg. Prazosin: 1.0 (○), 2.0 (●), and 4.0 (△) mg/kg. The numbers in the horizontal represent the same as Fig. 1. Each value represents the mean ± S.D. of eight animals. *$p<0.05$ vs. control value (baseline) by Dunnett's multiple comparison procedure.

dosing and at dose levels of 2.0 and 4.0 mg/kg over a period of 35 min. Vehicle alone did not show any significant effect over a period of 35 min.

Crotononitrile-Induced Dyskinesia

Figure 2 shows the effects of the drugs on the crotononitrile-induced behavioral abnormalities. Clonidine (0.1 and 0.2 mg/kg) inhibited the dyskinetic syndrome over a period of 35 min, while 0.05 mg did not produce any significant effect, $F(3,92)=9.99$, $p<0.01$. Phenylephrine (8.0 mg/kg) caused inhibition of the syndrome, while 2.0 and 4.0 mg/kg did not show any effect over an observation period of 35 min, $F(3,92)=11.67$, $p<0.01$. Yohimbine (2.0, 4.0 and 8.0 mg/kg) had no effect on the score. Prazosin (2.0 and 4.0 mg/kg) reduced the syndrome over a period of 35 min, whereas 0.1 mg/kg had no significant effect, $F(3,92)=12.77$, $p<0.01$. Vehicle alone had no effect.

2-Pentenenitrile-Induced Dyskinesia

Figure 3 shows the effects of the drugs on the 2-pentenenitrile-induced dyskinetic syndrome. Clonidine (0.2 mg/kg) blocked the syndrome, while 0.05 and 0.1 mg/kg had no effect on the dyskinesia score, $F(3,92)=13.88$, $p<0.01$. Phenylephrine (8.0 mg/kg) reduced the syndrome over a period of 35 min, but 2.0 and 4.0 mg/kg did not, $F(3,92)=11.96$, $p<0.01$. Yohimbine (2.0, 4.0 and 8.0 mg/kg) had no effect on the score. Prazosin inhibited the dyskinesia, $F(3,92)=13.88$, $p<0.01$, both at the dose level of 4.0 mg/kg over a period of 35 min and at the dose level of 2.0 mg/kg 30 to 35 min after dosing. No effect was observed 15 to 20 min after dosing (2.0 mg/kg) or over a period of 35 min (1.0 mg/kg). Vehicle alone had no effect.

DISCUSSION

A single administration of allylnitrile in mice can induce persistent dyskinetic syndrome consisting of random circling, head twitching, an increased locomotor activity and occasional backward running, the frequency increasing after one month and the syndrome lasting for at least 4 months (27). This situation is also true for crotononitrile and 2-pentenenitrile (28). The present observation was done two months after dosing with each nitrile. The drugs that blocked the dyskinetic syndrome induced by allylnitrile, crotononitrile and 2-pentenenitrile were the alpha$_2$-adrenoceptor agonist clonidine (20), the alpha$_1$-adrenoceptor agonist phenylephrine (13) and the alpha$_1$-adrenoceptor antagonist prazosin (18). The alpha$_2$-adrenoceptor antagonist yohimbine (19) had no effect on the dyskinesia.

The role of norepinephrine in behavioral control has been reported (15). Norepinephrine applied by microiontophoresis depresses the firing of neurons in the brain and its intraventricular infusion produced a general behavioral damping, suggesting the inhibitory action of norepinephrine. However, some conflicting results have also been reported (15); intraventricular injections of norepinephrine do not necessarily induce sedation, since small doses induce behavioral activation. It has been suggested that some neuronal systems using norepinephrine as a transmitter may be involved in behavioral arousal. There are two subtypes of alpha-adrenoceptor; the alpha$_1$-adrenoceptor as a postsynaptic receptor, and the alpha$_2$-adrenoceptor on noradrenergic nerve terminals (23), although postsynaptic alpha$_2$-receptors have also been reported (10).

The effects of clonidine and prazosin in this study were similar to those in 5-HT- and IDPN-induced dyskinesia, where clonidine and prozosin inhibited the head twitching induced by 5-HT and reduced the dyskinetic score of the IDPN-induced syndrome (5,13). The effect of clonidine is probably related to its ability to suppress locus cell firing (9). Alpha$_2$-adrenoceptor agonists are capable of producing sedation, but the inhibitory effect of clonidine does not appear to be due to sedation because this drug, at higher doses, does not cause any sedation in normal mice (13). Prazosin may exhibit its effect through blocking the postsynaptic alpha$_1$-adrenoceptors, suggesting that a tonic noradrenergic influence may be necessary in the occurrence of the syndrome.

The effects of phenylephrine were similar to those in a previous report (13) which showed that 2.5 to 10 mg/kg phenylephrine inhibited the head twitching induced by 5-HT and potentiated it at less than 2.0 mg/kg. The difference between the previous (13) and the present results might be due to the ceiling effect on the scale used, or to the dose level employed. It has been shown that yohimbine potentiates the 5-HT-induced head twitching (13) but has no effect on the IDPN-induced dyskinetic syndrome (5), the latter result being in accordance with the present data. The fact that yohimbine had no effect on the dyskinetic score might also be due to the ceiling effect on the scale used.

In this study, agents that have opposite neuropharmacologic effects on the noradrenergic systems had the same effect on the syndrome induced by each of the nitriles. Although the exact mechanism remains to be determined, the following interpretation of the results might be given based on the location of alpha$_1$- and

alpha$_2$-adrenoceptors in the synapse. Clonidine, an alpha$_2$-adrenoceptor agonist, inhibited the nitrile-induced dyskinesia and this effect may be due to stimulation of the postsynaptic alpha$_2$-adrenoceptors. It has been suggested (2) that alpha$_2$-adrenoceptors relevant to head twitching are not located presynaptically as alpha$_1$-adrenoceptors on the same synapse. Clonidine inhibition of the head twitching, induced by 5-hydroxytryptophan, is not blocked by the injection of 6-hydroxydopamine, indicating that the clonidine effect is not presynaptic (2). The fact that yohimbine, an alpha$_2$-adrenoceptor antagonist, had no effect in this study is probably due to the ceiling effect. Yohimbine has been reported to potentiate the 5-HT-induced head twitching and this effect is explained by the action on the postsynaptic alpha$_2$-adrenoceptors (13). In this study phenylephrine, an alpha$_1$-adrenoceptor agonist, had an inhibitory effect, and this may be due to alpha$_2$-adrenoceptor stimulation. Phenylephrine and methoxamine, alpha$_1$-adrenoceptor agonists, are known to inhibit the 5-HT-induced head twitching and the effect of methoxamine is reversed to potentiation in the presence of yohimbine (13). These facts suggest that the inhibition is due to alpha$_2$-adrenoceptor stimulation and that the stimulation of alpha$_1$-adrenoceptors results in behavioral potentiation. Prazosin, an alpha$_1$-adrenoceptor antagonist, had an inhibitory effect in this study, presumably by blocking the postsynaptic alpha$_1$-adrenoceptors.

The present results suggest that the noradrenergic system is involved in the manifestation of the syndrome induced by allylnitrile, crotononitrile and 2-pentenenitrile. Furthermore, a tonic noradrenergic influence on subcortical systems may be required in the occurrence of the syndrome induced by IDPN and each nitrile. Motor abnormalities produced by IDPN, resembling those by nitriles, were attenuated after lesions of the basal ganglia but not after cortical lesions (22), and the complete ablation of the frontal cortex failed to change the head shaking behavior induced by 5-HT precursor, 5-hydroxy-L-tryptophan, or by the serotonergic agonist, quipazin (17).

Crotononitrile, 2-pentenenitrile, allylnitrile and IDPN appear to share a common mechanism that induces a dyskinetic syndrome, in which the behavioral abnormalities persist. The present and previous (4, 5, 7, 22, 27, 28) studies have revealed that the separately induced syndrome respond in a similar manner to those drugs that have an affinity for alpha adrenoceptors, 5-HT receptors or dopamine receptors. In addition to IDPN these nitriles are useful when studying drugs that are effective in the treatment of human neuropsychiatric disorders and in the investigation of the etiology of dyskinesia.

ACKNOWLEDGEMENT

The authors acknowledge the secretarial help of Miss Chieko Demura.

REFERENCES

1. Arnt, J.; Hyttel, J.; Larsen, J-J. The citalopram/5-HTP-induced head shakes syndrome is correlated to 5-HT$_2$ receptor affinity and also influence by other transmitters. Acta Pharmacol. Toxicol. 55:363–372; 1984.
2. Bednarczyk, B.; Vetulani, J. Antagonism of clonidine to shaking behaviour in morphine abstinence syndrome and to head-twitches produced by serotonergic agents in the rat. Pol. J. Pharmacol. Pharm. 30:307–322; 1978.
3. Boulton, C. S.; Handley, S. L. Factors modifying the headtwitch response to 5-hydroxytryptophan. Psychopharmacologia 31:205–214; 1973.
4. Cadet, J. L.; Braun, T.; Freed, W. J. The dopamine D-2 antagonist, Ro 22-1319, inhibits the persistent behavioral syndrome by iminodipropionitrile (IDPN)-induced dyskinesia. Exp. Neurol. 96:594–600; 1987.
5. Cadet, J. L.; Braun, T.; Freed, W. J. Clonidine and prazosin block the iminodipropionitrile (IDPN)-induced spasmodic dyskinetic syndrome in mice. Pharmacol. Biochem. Behav. 26:791–795; 1987.
6. Cadet, J. L.; Jackson-Lewis, V.; Fahn, S. The iminodipropionitrile (IDPN) model of persistent spasmodic dyskinesias: regional serotonin metabolism in rat brain. Brain Res. 456:371–374; 1988.
7. Cadet, J. L. Participation of the serotonin$_2$ receptor in the iminodipropionitrile-induced dyskinetic syndrome. Exp. Neurol. 99:589–593; 1988.
8. Chou, S-M.; Hartmann, H. A. Axonal lesions and waltzing syndrome after IDPN administration in rats. With a concept-"axostasis." Acta Neuropathol. 3:428–450; 1964.
9. Cooper, J. R.; Bloom, F. E.; Roth, R. H. The biochemical basis of neuropharmacology. New York: Oxford Univ. Press; 1986:259–314.
10. Docherty, J. R.; McGrath, J. C. A comparison of pre- and postjunctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat: Evidence for two types of post-junctional alpha-adrenoceptor. Naunyn Schmiedebergs Arch. Pharmacol. 312:107–116; 1980.
11. Dunnett, C. W. New tables for multiple comparisons with a control. Biometrics 51:482–491; 1964.
12. Freed, W. J.; Bing, L. A.; Wyatt, R. J. Effects of neuroleptics on phencyclidine (PCP)-induced locomotor stimulation in mice. Neuropharmacology 23:175–181; 1984.
13. Handley, S. L.; Braun, J. Effects on the 5-hydroxytryptamine-induced head-twitch of drugs with selective actions on alpha$_1$- and alpha$_2$-adrenoceptors. Neuropharmacology 21:507–510; 1982.
14. Handley, S. L.; Singh, L. Modulation of 5-hydroxytryptamine-induced head-twitch response by drugs acting at GABA and related receptors. Br. J. Pharmacol. 86:297–303; 1985.
15. Iversen, S. D.; Iversen, L. L. Central neurotransmitters and the regulation of behavior. In: Gazzaniga, M. S.; Blakemore, G., eds. Handbook of psychobiology. New York: Academic Press; 1975: 153–200.
16. Jacobs, B. L. An animal model for studying central serotonergic synapses. Life Sci. 19:777–786; 1976.
17. Lucki, I.; Minugh-Purvis, N. Serotonin-induced head shaking behavior in rats does not involve receptors located in the frontal cortex. Brain Res. 420:403–406; 1987.
18. Miach, P. J.; Dausse, J. P.; Cardot, A.; Meyer, P. M. ^{3}H-Prazosin binds specifically to "alpha-1"-adrenoceptors in rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 312:23–26; 1980.
19. Perry, B. D.; U'Pritchard, D. C. [^{3}H]Rauwolscine (yohimbine): a specific antagonist radioligand for brain adrenergic receptors. Eur. J. Pharmacol. 76:461–464; 1981.
20. Rovot, B. R.; Snyder, S. H. [^{3}H]Para-aminoclonidine: a novel ligand which binds with high affinity to α-adrenergic receptors. Life Sci. 25:769–779; 1979.
21. Schneider, G.; Oepen, H.; Klapproth, A. The effect of the neurolathyrogenic substance, β,β′-iminodipropionitrile (IDPN) on some biological parameters in rats and mice. Gen. Pharmacol. 12:109–114; 1981.
22. Schneider, J. S. Role of the basal ganglia in a chemically induced dyskinesia in rat. Exp. Neurol. 84:524–532; 1984.
23. Starke, K. Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol. Biochem. Pharmacol. 77:1–124; 1977.
24. Sturgeon, R. D.; Fessler, R. G.; Meltzer, H. Y. Behavioral scale for assessing phencyclidine-induced locomotor activity, stereotyped behavior, and ataxia in rats. Eur. J. Pharmacol. 59:169–179; 1979.
25. Tanii, H.; Hashimoto, K. Studies on the mechanism of acute toxicity of nitriles in mice. Arch. Toxicol. 55:47–54; 1984.
26. Tanii, H.; Hashimoto, K. Structure-toxicity relationship of aliphatic nitriles. Toxicol. Lett. 22:267–272; 1984.
27. Tanii, H.; Kurosaka, Y.; Hayashi, M.; Hashimoto, K. Allylnitrile: A compound which induces long-term dyskinesia in mice following a single administration. Exp. Neurol. 103:64–67; 1989.
28. Tanii, H.; Hayashi, M.; Hashimoto, K. Nitrile-induced behavioral abnormalities in mice. Neurotoxicology 10:157–166; 1989.