

**Effect of Antidepressants on Locomotor Activity in Young Chicken<sup>1)</sup>**

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Effect of antidepressants on the locomotor activity in paired young chicks was examined in comparison with that of other psychotropic drugs. Tricyclic antidepressants, imipramine, chlorimipramine, amitriptyline and nortriptyline caused an increase in locomotor activity together with hyperactivity. PF-82 and cocaine, uptake inhibitors, also caused a similar increase of the locomotor activity and behavioral changes to those of tricyclic antidepressants. On the other hand, behavioral effects of pargyline, iproniazid, methamphetamine, diazepam and chlordiazepoxide were different from those of tricyclic antidepressants. Chlorpromazine increased the locomotor activity in a manner similar to that of imipramine. Phentolamine,  $\alpha$ -adrenergic blocking agent, however, caused little or no effects on the locomotor activity and behavioral changes. The locomotor activity increased by imipramine or chlorpromazine was inhibited by phentolamine. These results suggest an involvement of the uptake inhibition of central adrenergic neurones in connection with the increase in chick locomotor activity.

Imipramine and other tricyclic antidepressants have been widely used as therapeutic drugs for mental depression.<sup>3)</sup> In animal experiments, it has been demonstrated that the mechanism of the main pharmacological effect of these drugs was the potentiation of the central action of noradrenaline by blocking the presynaptic reuptake.<sup>4)</sup> However, these antidepressants are known to have, in general, no effects on the behavior of mammals when given alone.<sup>5)</sup>

In contrast to those effects in mammals, it was reported that imipramine markedly increased the locomotor activity of young chicks with immature blood brain barrier.<sup>6)</sup> The same effect was confirmed by Schrold<sup>7)</sup> with several tricyclic antidepressants. In the present study, the influence on behavior was examined in chicks not only with antidepressants but also with other psychotropic drugs. Special attention was attached to the locomotor activity to clarify whether or not the stimulating effect in chicks is specific for antidepressants.

As a result obtained, chlorpromazine was found to cause a marked increase in locomotor activity in a manner similar to that of imipramine. Mandell, *et al.*<sup>6)</sup> suggested that the increase in chick locomotor activity induced by imipramine was due to the postsynaptic blockage of the central noradrenergic receptor. However, the effect of imipramine has been explained as the result of the uptake inhibition. Also, it has been reported that chlorpromazine caused not only the postsynaptic blockage but also the uptake inhibition.<sup>8)</sup> Therefore, a possibility that the increase in locomotor activity may occur through the uptake inhibition of noradrenaline can not be discarded. In an attempt to clarify the mechanism underlying this pheno-

- 1) This work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April, 1974.
- 2) Location: *Enoki-cho, Suita, Osaka, 564, Japan.*
- 3) J.H. Biel, "Drugs Affecting the Central Nervous System 2," ed. by A. Burger, Arnold, London, 1968, p. 61.
- 4) J. Glowinski and J. Axelrod, *Nature, Lond.*, **204**, 1318 (1964).
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- 6) A.J. Mandell, C.E. Spooner, W.D. Winters, M. Cruikshank and I.M. Sabbot, *Life Sciences*, **7**, 1317 (1968); *idem*, *Int. J. Neuropharmac.*, **8**, 235 (1969).
- 7) J. Schrold, *Psychopharmacologia* (Berl.), **17**, 225 (1970).
- 8) H.J. Dengler and E.O. Titus, *Biochem. Pharmacol.*, **8**, 64 (1961).

menon, the effect of phentolamine,  $\alpha$ -adrenergic blocking agent, and the interaction between phentolamine and either imipramine or chlorpromazine were also examined.

### Materials and Methods

The experiments were performed on male young chicks (White Leghorn strain, weighing 40–60 g) ranging in age from 2 to 6 days after hatching. Two chicks were used in pairs as described by Schrold.<sup>7)</sup> After the intraperitoneal injection of drugs in a volume of 2.0 ml/kg, the chicks were placed in an uncovered plastic cage (36 × 21 × 30 cm) on an "Animex" activity meter (Farad Electronics, DS). Locomotor activity was measured in counts per 20 min for 100 min, and behavioral change was observed at the same time. Chicks were kept in an air-conditioned room at  $25 \pm 1^\circ$  with a  $65 \pm 5\%$  relative humidity.

Drugs used were imipramine-HCl, amitriptyline-HCl, chlorimipramine-HCl, nortriptyline-HCl, methamphetamine-HCl, pargyline-HCl, iproniazid phosphate, PF-82 (3-(N-3,3-diphenylpropyl-N-methyl)-amino-propan-1-ol-HCl),<sup>9)</sup> cocaine-HCl, chlorpromazine-HCl, haloperidol, oxypertine-HCl, diazepam, chlordiazepoxide and phentolamine-HCl. These were dissolved with 0.9% saline or suspended with 0.5% tragacanth solution.

### Results

#### Tricyclic Antidepressants

Imipramine-HCl (5 mg/kg, *i.p.*) increased the locomotor activity: 1140, 1220 and 1140 counts/20 min in 20–40, 40–60 and 60–80 min after the injection, respectively (Fig. 1A). These counts were significantly different from those of the control. The chicks vocalized

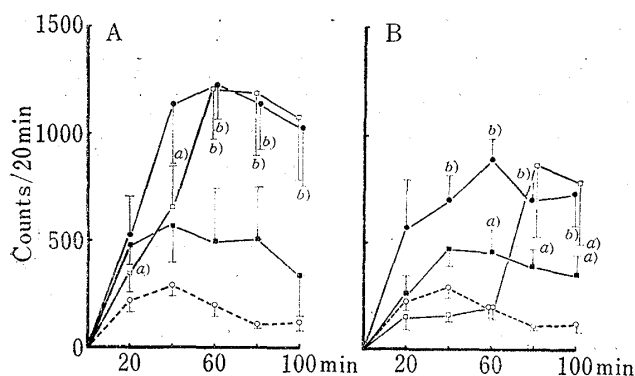


Fig. 1. Effect of Imipramine and Amitriptyline on the Locomotor Activity in Paired Chicks

abscissa: time after the injection, ordinate: locomotor activity in counts per 20 min

A: imipramine-HCl, —■—: 1 mg/kg, —●—: 5 mg/kg, —□—: 12.5 mg/kg, —○—: control (tragacanth).

B: amitriptyline-HCl, —■—: 1 mg/kg, —●—: 5 mg/kg, —□—: 50 mg/kg, —○—: control (tragacanth).

Each point represents the mean counts in separate five experiments with the S.E. indicated.

a)  $p < 0.05$  and b)  $p < 0.01$  as compared with the control.

(twittering), sometimes aggressively pecked each other's legs or eyes (attack pecking), and tapped repeatedly with their legs on the floor (repetitive tapping). The increase in locomotor activity appeared even at a low dose of 1 mg/kg. Amitriptyline-HCl (5 mg/kg) also increased the locomotor activity (Fig. 1B). However, at a high dose of amitriptyline-HCl (50 mg/kg) sedation was observed for approximately 60 min after the injection.

Figs. 2 and 3 show the comparison of the changes in locomotor activity of the drug-treated groups with those of the control group, represented by the total mean counts during 100 min after the injection. The total mean counts of the locomotor activity induced by 5 mg/kg of imipramine-HCl were 4–5 times more

than those of the control. Other tricyclic antidepressants, amitriptyline, chlorimipramine and nortriptyline showed almost the same activity as imipramine. Schrold<sup>7)</sup> reported that aggressiveness always occurred together with hyperactivity after the injection of tricyclic antidepressants. In the present experiments, however, the increase of attack pecking behavior did not consistently occur.

#### Monoamine Oxidase Inhibitors and Methamphetamine

Pargyline-HCl (10 and 25 mg/kg) and iproniazid phosphate (10 and 25 mg/kg) showed little or no effects on the locomotor activity and behavior. Methamphetamine-HCl (5 mg/kg)

9) M. Shimizu, T. Hirooka, T. Karasawa, Y. Masuda, M. Oka, T. Ito, C. Kamei, Y. Sohji, M. Hori, K. Yoshida and H. Kaneko, *Arzneimittel-Forsch. (Drug Res.)*, **24**, 166 (1974).

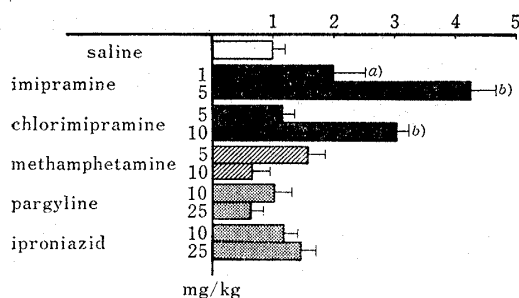


Fig. 2. Effect of Psychotropic Drugs on the Locomotor Activity in Paired Chicks

abscissa: ratios of the total mean counts (100 min) of drug-treated groups to those of the control (saline control=1).

a)  $p < 0.05$  and b)  $p < 0.01$  as compared with the control.

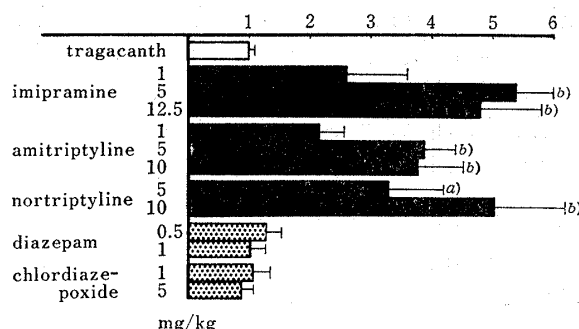


Fig. 3. Effect of Psychotropic Drugs on the Locomotor Activity in Paired Chicks

abscissa: ratios of the total mean counts (100 min) of drug-treated groups to those of the control (tragacanth control=1).

a)  $p < 0.05$  and b)  $p < 0.01$  as compared with the control.

showed an increase in alertness level such as open eyelids, twittering and attack pecking. In spite of these behavioral changes, the total mean counts of the locomotor activity was not significantly different from that of the control (Fig. 2). With a dose of 10 mg/kg of methamphetamine-HCl, the locomotor activity was decreased, and the behavioral change from standing to prone and wing dropping was induced.

### Diazepam and Chlordiazepoxide

Diazepam (0.5 and 1.0 mg/kg) and chlordiazepoxide (1 and 5 mg/kg) showed little or no effect on the locomotor activity (Fig. 3), while higher doses of these drugs caused a decrease of muscle tone.

### PF-82 and Cocaine

PF-82<sup>9)</sup> and cocaine were used as drugs which possess the property of inhibiting noradrenaline reuptake. PF-82 (5 mg/kg) gradually increased the locomotor activity: 840, 868 and 1010 counts/20 min in 40—60, 60—80 and 80—100 min after the injection, respectively (Fig. 4). The increasing effect with PF-82 appeared more slowly as compared with that of imipramine.

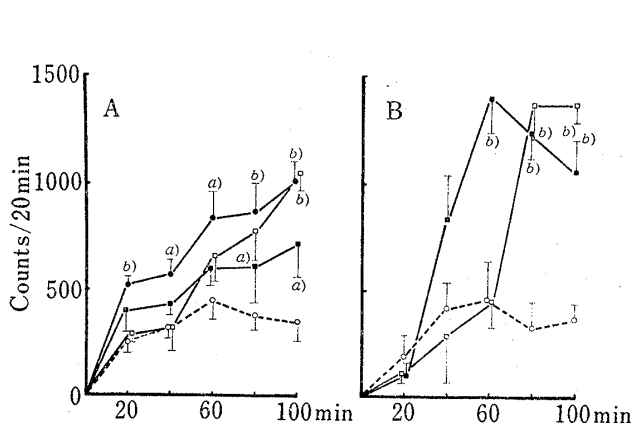


Fig. 4. Effect of PF-82 and Cocaine on the Locomotor Activity in Paired Chicks

abscissa: time after the injection, ordinate: locomotor activity in counts per 20 min

A: PF-82, —■—: 1 mg/kg, —●—: 5 mg/kg, —□—: 25 mg/kg, ---○---: control (saline)

B: cocaine-HCl, —■—: 10 mg/kg, —□—: 25 mg/kg, ---○---: control (saline) Each point represents the mean counts in separate five experiments with the S.E. indicated.

a)  $p < 0.05$  and b)  $p < 0.01$  as compared with the control.

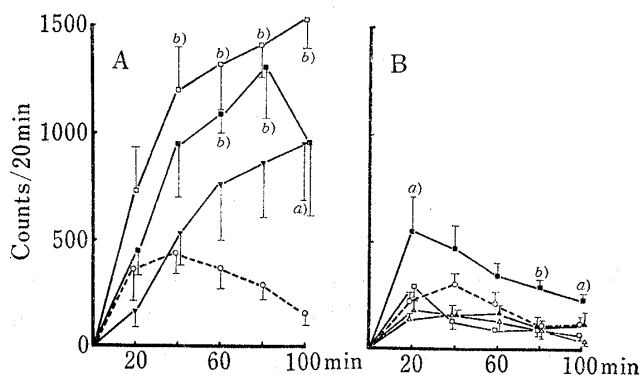


Fig. 5. Effect of Chlorpromazine, Haloperidol and Oxypertine on the Locomotor Activity in Paired Chicks

abscissa: time after the injection, ordinate: locomotor activity in counts per 20 min

A: chlorpromazine-HCl, —□—: 5 mg/kg, —■—: 10 mg/kg, —▼—: 25 mg/kg, ---○---: control (saline)

B: haloperidol, —■—: 5 mg/kg, —□—: 10 mg/kg, oxypertine-HCl, —▲—: 15 mg/kg, —△—: 30 mg/kg, ---○---: control (tragacanth)

Each point represents the mean counts in separate five experiments with the S.E. indicated.

a)  $p < 0.05$  and b)  $p < 0.01$  as compared with the control.

Cocaine-HCl (10 mg/kg) markedly increased the locomotor activity: 1400 and 1240 counts/20 min in 40–60 and 60–80 min after the injection, respectively (Fig. 4). These counts were significantly different from those of the control. In the administrated dose-range both drugs caused behavioral changes such as twittering, repetitive tapping and attack pecking. These effects were similar to those of tricyclic antidepressants.

### Chlorpromazine, Haloperidol and Oxypertine

Chlorpromazine-HCl (5 mg/kg) caused a marked increase in locomotor activity: 730, 1200 and 1530 counts/20 min in 0–20, 20–40 and 80–100 min after the injection, respectively (Fig. 5). Behavioral changes such as moving around and attack pecking were induced. A higher dose of chlorpromazine-HCl (25 mg/kg) induced sedative state such as sitting on the shanks and lying down with its head on floor for 40 min after the injection, followed by hyperactivity and aggressiveness. The increase in locomotor activity was less than that observed with 5 mg/kg. Haloperidol (5 mg/kg) caused a slight increase in locomotor activity while at a high dose (10 mg/kg) a slight decrease occurred. Oxypertine-HCl (15 and 30 mg/kg) slightly decreased the locomotor activity.

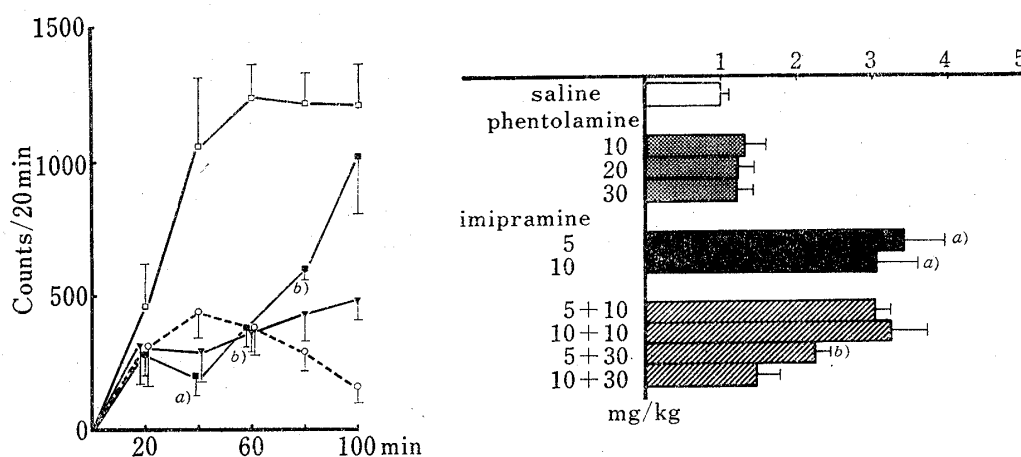


Fig. 6. Interaction between Phentolamine and Imipramine on the Locomotor Activity in Paired Chicks

left graph: effect on the locomotor activity in counts per 20 min  
 —□—: imipramine-HCl 5 mg/kg, —○—: phentolamine-HCl 30 mg/kg, —■—: simultaneous injection of imipramine-HCl 5 mg/kg and phentolamine-HCl 30 mg/kg, —○—: control (saline)  
 Each point represents the mean counts in separate five experiments with the S.E. indicated.  
 a)  $p < 0.05$  and b)  $p < 0.01$  as compared with the effect of imipramine.  
 right graph: ratios of the total mean counts (100 min) of drug-treated groups to those of the control (saline control=1).  
 a)  $p < 0.01$  as compared with the control. b)  $p < 0.01$  as compared with the effect of imipramine.

### Interaction between Phentolamine and either Imipramine or Chlorpromazine

Phentolamine-HCl caused no effects on the locomotor activity and behavior in a dose range of 10–30 mg/kg (Fig. 6). With a simultaneous injection of imipramine-HCl (5 mg/kg) and phentolamine-HCl (30 mg/kg), the mean counts of the locomotor activity were 200, 381 and 600 counts/20 min in 20–40, 40–60 and 60–80 min after the injection, respectively (Fig. 6). These counts were significantly different from those of 5 mg/kg of imipramine-HCl. During the period, twittering, repetitive tapping and attack pecking behaviors did not appear. The result indicates that the increase in locomotor activity induced by imipramine was inhibited by phentolamine.

With a simultaneous injection of chlorpromazine-HCl (5 mg/kg) and phentolamine-HCl (10 mg/kg), the mean counts of the locomotor activity were 81, 530, 676 and 840 counts/20 min in 0–20, 20–40, 60–80 and 80–100 min after the injection, respectively (Fig. 7). These counts were significantly different from those of 5 mg/kg of chlorpromazine-HCl. The pattern

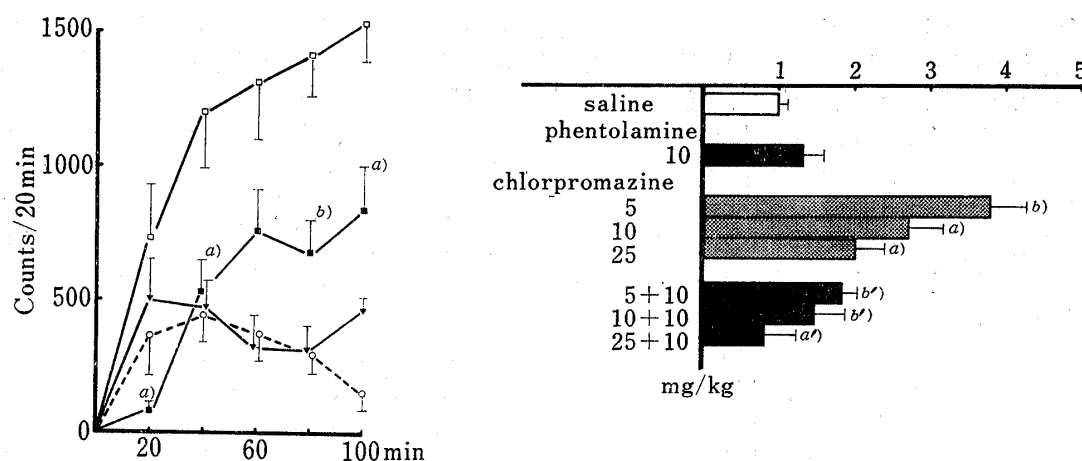


Fig. 7. Interaction between Phentolamine and Chlorpromazine on the Locomotor Activity in Paired Chicks

left graph: effect on the locomotor activity in counts per 20 min  
 —□—: chlorpromazine-HCl 5 mg/kg, —▼—: phentolamine-HCl 10 mg/kg, —■—: simultaneous injection of chlorpromazine-HCl 5 mg/kg and phentolamine-HCl 10 mg/kg, ---○---: control (saline).  
 Each point represents the mean counts in separate five experiments with the S.E. indicated.  
 a)  $p < 0.05$  and b)  $p < 0.01$  as compared with the effect of chlorpromazine.  
 right graph: ratios of the total mean counts (100 min) of drug-treated groups to those of the control (saline control=1).  
 a)  $p < 0.05$  and b)  $p < 0.01$  as compared with the control.  
 a')  $p < 0.05$  and b')  $p < 0.01$  as compared with the effect of chlorpromazine.

of response was similar to that of chlorpromazine-HCl 25 mg/kg; postural changes such as sitting and lying down were observed 20 min after the injection. The increase in locomotor activity induced by chlorpromazine was inhibited by phentolamine.

### Discussion

As shown in Figs. 1—3, tricyclic antidepressants examined caused an increase in locomotor activity of paired young chicks; the total mean counts (during 100 min) of the locomotor activity induced by imipramine (5 mg/kg), amitriptyline (5 mg/kg), chlorimipramine (10 mg/kg) or nortriptyline (10 mg/kg) were 3—6 times more than those of the control. The present result coincided with that reported by Schrold.<sup>7)</sup> With these drugs, behavioral changes characterized mainly by twittering, repetitive tapping and increased attack pecking were observed. On the other hand, pargyline, iproniazid, methamphetamine, diazepam and chlordiazepoxide did not cause a marked increase in chick locomotor activity. These drugs except for methamphetamine produced little or no behavioral changes. PF-82 and cocaine,<sup>10)</sup> having chemical structures different from tricyclic antidepressants and possessing the property to inhibit noradrenaline reuptake, caused almost the same increase in locomotor activity and behavioral changes as tricyclic antidepressants. It seems likely that the drug with the property to inhibit reuptake causes an increase in locomotor activity of paired young chicks.

Methamphetamine (5 mg/kg) slightly increased the locomotor activity and caused behavioral changes such as twittering and attack pecking, while a higher dose (10 mg/kg) of methamphetamine decreased the locomotor activity and caused wing dropping. These behavioral changes were similar to those of amphetamine reported by Schrold, *et al.*<sup>11)</sup> and Wallach, *et al.*<sup>12)</sup> It has been indicated that amphetamine acted in the brain through releasing noradrenaline from nerve terminals.<sup>13)</sup> Also, a monoamine oxidase inhibitor has been reported

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- 11) J. Schrold and R.F. Squires, *Psychopharmacologia* (Berl.), **20**, 85 (1971).
- 12) M.B. Wallach, E. Friedman and S. Gershon, *Europ. J. Pharmacol.*, **17**, 259 (1972).
- 13) N.E. Andén, "Amphetamine and Related Compounds," Proceedings of the Mario Negri Institute of Pharmacological Research, Milan, ed. by E. Costa and S. Garattini, Raven Press, New York, 1970, p. 447.

to block monoamine oxidase and cause a subsequent rise in the intra- and extra-neuronal concentration of noradrenaline.<sup>14)</sup> Thus, the similarities seem to exist in the actions of antidepressants, methamphetamine and monoamine oxidase inhibitors in so far as an increase in the catecholamine concentration occurs at the synaptic cleft. In this experiment, however, the differences in actions were observed on the chick locomotor activity among these drugs, suggesting that other neuronal mechanisms activated or inhibited by the drugs seem to be related with the locomotor activity. In this aspect, it was recently reported that antidepressants antagonized the behavioral changes induced by *d*-amphetamine in chicks, through a predominant serotonergic mechanism.<sup>15)</sup>

In the present experiments, a low dose of chlorpromazine was found to cause an increase in chick locomotor activity. The result supports partially the view of Spooner, *et al.*<sup>16)</sup>; they indicated that chicks were more resistant to the depressant effects of chlorpromazine than mammals. As stated previously, one of the purpose of this study was to clarify the mechanism underlying the stimulating effects in chicks. Phentolamine which inhibits  $\alpha$ -adrenergic neurones caused little or no effects on the locomotor activity and behavioral changes. This fact implies that the postsynaptic block may not be involved with the increase in locomotor activity. Also, the increase in locomotor activity and behavioral changes induced by either chlorpromazine or imipramine were inhibited by phentolamine. The results indicate the existence of common mechanisms between actions of imipramine and chlorpromazine, and a possibility that phentolamine interferes with the action of these drugs at postsynaptic sites of the central adrenergic neurones.

From the results described above, it is suggested that the increase in chick locomotor activity is, in part, attributed to the reuptake inhibition of central adrenergic neurones. In addition, this method is available as a simple test evaluating an antidepressant activity since the stimulating effect in chicks could be obtained by antidepressants with the uptake inhibition of noradrenaline which is thought to play a significant role in the clinical antidepressant activity.

**Acknowledgement** The authors express their thanks to Miss Y. Shiraishi for her excellent technical assistance.

14) L.I. Goldberg, *J.A.M.A.*, **190**, 456 (1964).

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