[^{3}H]dopamine in striatal slices of mice (Ross & Renyi, 1975) and rats (ED50 = 67 mg kg^{-1}, i.p. 0·5 h after the injection, unpublished observation). Furthermore, methylphenidate produces stereotypies in normal (non-reserpinized) rats at the same doses (20 mg kg^{-1}, i.p. and more; cf. Scheel-Krüger, 1971) as those antagonizing the stereotypies produced by (+)-amphetamine in reserpinized rats. Other findings are also in accordance with the hypothesis that methylphenidate is an inhibitor of the dopamine uptake *in vivo*. Thus, the antagonism by reserpine of the stimulatory effect of methylphenidate is explained by the failure of the impulse propagated release of dopamine. Shore (1976) observed that methylphenidate + haloperidol lowers the striatal dopamine in α-methyltyrosine treated rats, which can be explained by the increased impulse flow combined with the inhibition of the re-uptake of dopamine. The stimulants of the methylphenidate group cause less tolerance than those of the amphetamine group (Biel, 1970), which is understood by the proposed difference in the mode of action between these goups of stimulants.

November 11, 1977

REFERENCES

BIEL, J. H. (1970). In: *Amphetamine and related compounds*, pp. 3–19. Editors: Costa, E. & Garattini, S., New York: Raven Press.

CARLSSON, A., FUXE, K., HAMBERGER, B. & LINDQVIST, M. (1966). *Acta physiol. scand.*, **67**, 481–497.

COSTALL, B., NAYLOR, R. J. & OLLEY, J. E. (1972). *Eur. J. Pharmac.*, **18**, 83–94.

ERNST, A. M. (1965). *Psychopharmac.*, **7**, 391–399.

FARNEBO, L-O. (1971). *Acta physiol. scand.*, **371**, *Suppl.*, 45–52.

FERRIS, R. M., TANG, F. L. M. & MAXWELL, R. A. (1972). *J. Pharmac. exp. Ther.*, **181**, 407–416.

PATON, D. M. (1974). *Am. Heart J.*, **88**, 128–129.

ROSS, S. B. (1976). In: *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines*, pp. 67–93. Editor: Paton, D. M. New York: Raven Press.

ROSS, S. B. (1977a). *J. Pharm. Pharmac.*, **29**, 433–434.

ROSS, S. B. (1977b). *Acta pharmac. tox.*, **41**, 392–396.

ROSS, S. B. & RENYI (1975). *Ibid.*, **36**, 56–66.

SCHEEL-KRÜGER, J. (1971). *Eur. J. Pharmac.*, **14**, 47–59.

SHORE, P. A. (1976). *J. Pharm. Pharmac.*, **28**, 855–857.

TRENDELENBURG, U. (1972). In: *Catecholamines*, pp. 336–362. Editors: Blaschko, H. & Muscholl, E. Berlin: Springer.

Effects of reserpine, *para*-chlorophenylalanine, 5,6-dihydroxytryptamine and fludiazepam on the head twitches induced by 5-hydroxytryptamine or 5-methoxytryptamine in mice

MITSUTAKA NAKAMURA*, HIDEAKI FUKUSHIMA, *Research and Development Center, Pharmaceuticals Division, Sumitomo Chemical Co., Ltd., 4-2-1, Takatsukasa, Takarazuka, Hyogo, 665, Japan*

Reserpine decreases the concentration of brain catecholamines in rodents. Many authors have reported that behavioural activities of noradrenaline (intraventricularly) or apomorphine, a dopamine receptor agonist, are enhanced after treatment with reserpine (Rotrosen, Angrist & others, 1972; Geyer & Segal, 1973; Symes, Lal & others, 1977). These observations have been taken as evidence that pretreatment with reserpine results in increased catecholamine receptor activity. Moreover, chronic depletion of catecholamines following inhibition of their synthesis and destruction of nerve terminals produced an increase in catecholamine receptor activity (Dominic & Moore, 1969; Thornburg & Moore, 1973). On the other hand, destruction of central 5-HT nerve terminals with intraventricular 5,6-dihydroxytryptamine (5,6-DHT) produces supersensitivity to 5-HT precursors and agonists (Nygren, Fuxe & others, 1974; Nakamura & Fukushima, 1978). Chronic inhibition of 5-HT synthesis with *p*-chlorophenylalanine (*p*CPA) also produces supersensitivity to 5-HT agonists (Przegalinski, Zebrowska & others, 1976). Trulson, Eubanks & Jacobs (1976), however, reported that supersensitivity to 5-HT precursors and agonists occurs following destruction of central 5-HT nerve terminals with 5,7-dihydroxytryptamine (5,7-DHT), but no supersensitivity occurs following chronic 5-HT depletion with *p*CPA.

It has been shown that head twitches are due to increased activity of 5-HT neuron systems (Corne, Pickering & Warner, 1963; Nakamura & Fukushima, 1976; Nakamura, Fukushima & Kitagawa, 1976; Nakamura & Fukushima, 1977, 1978). Moreover, head twitches have been used to study 5-HT neuron activity. There are several drugs that induce head twitches in mice; 5-HT (intracerebrally; i.c.) and 5-hydroxytryptophan by increasing the free concentration of 5-HT

* Correspondence.

at its receptor site (Corne & others, 1963; Nakamura & others, 1976), 5-methoxytryptamine (5-MT) by directly mimicking 5-HT at its receptors (Przegalinski & others, 1976), and benzodiazepines by increasing 5-HT receptor activity (Nakamura & Fukushima, 1976, 1977, 1978). In the present study we have compared the effect of pretreatment with reserpine or *p*CPA on the head twitches induced by 5-HT (i.c.) or 5-MT (i.v.) in mice with that of 5,6-DHT and fludiazepam, one of the benzodiazepines which are known to cause an increase in 5-HT receptor activity (Nygren & others, 1974; Nakamura & Fukushima, 1977, 1978).

Ten male dd strain mice, 20–22 g, were used in each group. All experiments were performed in an illuminated room with a 12 h daylight cycle and a relatively constant environment (24 ± 1° and 55 ± 5% humidity). Fludiazepam and *p*CPA were suspended in 5% arabic gum and 0·5% carboxymethylcellulose solution, respectively. Reserpine, 5-MT·HCl and 5-HT creatinine sulphate were dissolved in saline. Reserpine (2·5 mg kg^{-1}, i.p.) was injected at 1, 3, 5, 7, 12, 24, 48 and 72 h before the injection of 5-HT (10 μg, i.c.). Fludiazepam (3 mg kg^{-1}) was given to mice orally 1 h before the injection of 5-MT (i.v.). 5,6-DHT creatinine sulphate (50 μg free base), dissolved in 20 μl of isotonic saline solution (with 0·1% ascorbic acid) at 4°, was injected intracerebrally under light ether anaesthesia within 10 s 12 days before the injection of 5-MT. *p*CPA (400 mg kg^{-1}, i.p.) was given to mice at 6 and 30 h before injection of 5-MT. Numbers of head twitches were counted for 2 min in white light, after transferring the mice singly to a plastic box (12 cm long × 18 cm high × 12 cm wide). The concentration of 5-HT in the brain was determined in aliquots of two pooled brains by the method of Curzon & Green (1971).

Reserpine, 2·5 mg kg^{-1}, caused a decrease in the concentration of brain 5-HT, that reached a maximum 24 h after the reserpine. Head twitches induced by 5-HT (i.c.) were potentiated by pretreatment with reserpine. This potentiation reached a maximum at 7 h after injection of reserpine (Fig. 1). 5-MT also induced head twitches in mice in a dose-dependent manner (Table 1). Head twitches induced by 5-MT were markedly potentiated by pretreatment with reserpine (2·5 mg kg^{-1}, at 7 h before 5-MT) or by *p*CPA (400 mg kg^{-1}). Pretreatment with *p*CPA depleted the concentration of brain 5-HT. The number of head twitches induced by 5-MT was also significantly increased in 5,6-DHT treated mice. The concentration of brain 5-HT in 5,6-DHT treated mice was 64% of that in control mice. Pretreatment with fludiazepam significantly increased the number of head twitches induced by 5-MT. However, fludiazepam at a dose of 3 mg kg^{-1} did not significantly change the concentration of brain 5-HT.

It has been shown that the relatively long-term depletion of presynaptic catecholamines may result in hypersensitive post-synaptic receptors (Rotrosen & others, 1972; Geyer & Segal, 1973; Symes & others,

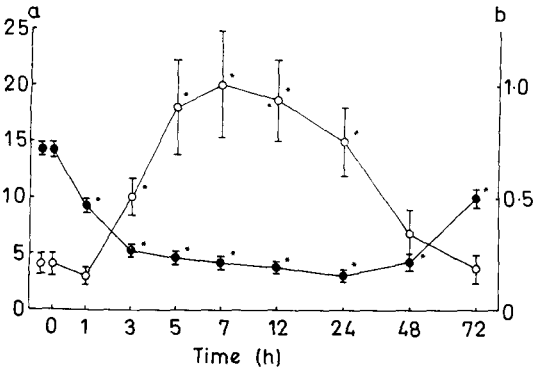

FIG. 1. Effect of reserpine on a: head twitches induced by 5-HT (10 μg, i.c.) (○—○) and b: the concentration of brain 5-HT (●—●) in mice. 5-HT was injected intracerebrally at various times after reserpine (2·5 mg kg^{-1}, i.p.). Control mice were treated with saline instead of reserpine. Head twitches were counted from 8–10 min after 5-HT. Vertical bars represent the standard error of the mean. * $P < 0.05$ when compared to control group (Student's *t*-test). Ordinates: a: Number of head twitches; b: Brain 5-HT (μg g^{-1} tissue). Abscissa: Time after reserpine (h).

1977). Pretreatment with reserpine markedly potentiated the head twitches induced by 5-HT (i.c.) in the present experiments. Injection of noradrenaline (up to 30 μg, i.c.), dopamine (up to 30 μg, i.c.), apomorphine (up to 50 mg kg^{-1}, i.p.) and clonidine (up to 2 mg kg^{-1}, i.p.), an α-adrenoceptor agonist, did not induce head twitches in normal and reserpinized mice (unpublished data). Therefore the potentiation of reserpine on head twitches induced by 5-HT (i.c.) might not be involved in the increase in catecholamine receptor activity. The number of head twitches induced by 5-MT was also markedly increased by pretreatment with reserpine. Przegalinski & others (1976) reported that head twitches induced by 5-MT were due to its direct stimulation of central 5-HT receptors. From these results, we suggest that 5-HT receptor activity is increased following the treatment with reserpine.

*p*CPA depleted the concentration of brain 5-HT in mice. The number of head twitches induced by 5-MT was increased by pretreatment with *p*CPA. This result was consistent with the finding of Przegalinski & others (1976) who showed that pretreatment with *p*CPA potentiated head twitches induced by 5-MT in rats. Thus chronic inhibition of 5-HT synthesis with *p*CPA produced an increase in 5-HT receptor activity. The present finding, however, is inconsistent with the report of Trulson & others (1976) who reported that 5-methoxy-*N*,*N*-dimethyltryptamine, a direct 5-HT agonist, did not elicit a supersensitive response in rats pretreated with *p*CPA. This different result might be due to the difference between the two models which were used to examine the activity of central 5-HT neurons.

Table 1. *Effects of reserpine, 5,6-dihydroxytryptamine (5,6-DHT), para-chlorophenylalanine (pCPA) and fludiazepam on head twitches induced by 5-methoxytryptamine (5-MT) in mice.*

Drugs	Number of head twitches per mouse ± s.e. (mg kg^{-1} of 5-MT)					Brain 5-HT ± s.e.
	0	5	10	15	20	(μg g^{-1} tissue)
Saline	0·3 ± 0·2	0·2 ± 0·2	0·5 ± 0·3	2·1 ± 0·5	4·3 ± 1·2	0·70 ± 0·03
Reserpine	0·6 ± 0·4	1·8 ± 0·5*	4·6 ± 0·8*	10·4 ± 1·8*		0·25 ± 0·02*
*p*CPA	0·5 ± 0·3	1·6 ± 0·4*	3·9 ± 0·7*	9·2 ± 2·2*		0·18 ± 0·01*
5,6-DHT	0·4 ± 0·4	3·0 ± 0·7*	9·4 ± 1·5*	18·2 ± 3·6*		0·45 ± 0·03*
Fludiazepam	0·6 ± 0·4	1·4 ± 0·5*	3·2 ± 0·6*	8·3 ± 1·5*		0·71 ± 0·04

Reserpine (2·5 mg kg^{-1}, i.p.), 5,6-DHT (50 μg, i.c.) and fludiazepam (3 mg kg^{-1}, orally) were administered to mice at 7 h, 12 days and 1 h, respectively, before injection of 5-MT (i.v.). *p*CPA (400 mg kg^{-1}, i.p.) was injected into mice at 6 and 30 h before injection of 5-MT. Head twitches were counted for 2 min immediately after 5-MT. * $P<0·05$ when compared to saline group (Student's *t*-test).

5,6-DHT produced a relatively selective chemical lesion of 5-HT nerve terminals and decreased the concentration of 5-HT (Baumgarten, Bjorklund & others, 1971). The injection of 5-MT to mice pretreated with 5,6-DHT caused a significant increase in the number of head twitches; a result consistent with the previous finding that degeneration of 5-HT nerve terminals produced by 5,6-DHT caused 5-HT receptor supersensitivity in mice (Nakamura & Fukushima, 1978).

Fludiazepam increased the number of head twitches induced by 5-MT. But fludiazepam did not change the concentration of brain 5-HT at the dose of 3 mg kg^{-1} which significantly potentiated the head twitches induced by 5-MT. These results are consistent with the previous suggestion that fludiazepam produced an increase in 5-HT receptor activity (Nakamura & Fukushima, 1976, 1977, 1978). Recently, Collier, Hammond & Schneider (1976) reported that head twitches were intensified by a change in favour of cyclic GMP of the balance between this nucleotide and AMP. But Costa, Guidotti & others (1976) reported that diazepam, one of the benzodiazepines, lowered the cerebellar GMP concentration, but did not change the concentration of cerebellar cAMP. Thus we think it is unlikely that fludiazepam potentiates the head twitches induced by 5-MT by a change of these nucleotide concentrations.

It is therefore suggested that reserpine, *p*CPA and 5,6-DHT deplete presynaptic 5-HT concentration and results in increased 5-HT receptor activity, whereas fludiazepam increases 5-HT receptor activity without affecting the concentration of brain 5-HT.

The authors express appreciation to Dr H. Yamamoto for his advice.

September 14, 1977

REFERENCES

BAUMGARTEN, H. G., BJORKLUND, A., LACHENMYER, L., NOBIN, A. & STENEVI, V. (1971). *Acta physiol. scand.*, **373**, Suppl., 1–15.

COLLIER, H. O. J., HAMMOND, M. D. & SCHNEIDER, C. (1976). *Br. J. Pharmac.*, **58**, 9–16.

CORNE, S. J., PICKERING, R. W. & WARNER, B. J. (1963). *Ibid.*, **20**, 106–120.

COSTA, E., GUIDOTTI, A., MAO, C. C. & SURIA, A. (1976). *Life Sci.*, **17**, 167–186.

CURZON, G. & GREEN, A. R. (1971). *Br. J. Pharmac.*, **39**, 653–654.

DOMINIC, J. A. & MOORE, K. E. (1969). *Psychopharmac.*, **15**, 96–101.

GEYER, M. A. & SEGAL, D. S. (1973). *Ibid.*, **29**, 131–140.

NAKAMURA, M. & FUKUSHIMA, H. (1976). *Ibid.*, **49**, 259–261.

NAKAMURA, M. & FUKUSHIMA, H. (1977). *Ibid.*, **53**, 121–126.

NAKAMURA, M. & FUKUSHIMA, H. (1978). *J. Pharm. Pharmac.*, **30**, 56–58.

NAKAMURA, M., FUKUSHIMA, H. & KITAGAWA, S. (1976). *Psychopharmac.*, **48**, 101–104.

NYGREN, L. G., FUXE, K., JONSSON, G. & OLSON, L. (1974). *Brain Res.*, **78**, 377–394.

PRZEGALINSKI, E., ZEBROWSKA, L. I., WOJCIK, A. & KLEINROK, Z. (1976). *Naunyn-Schmiedebergs Arch. Pharmac., Suppl.*, p. 14.

ROTROSEN, J., ANGRIST, B. M., WALLACH, W. B. & GERSHON, S. (1972). *Eur. J. Pharmac.*, **20**, 133–135.

SYMES, A. L., LAL, S., YOUNG, S. N., TSANG, D. & SOURKES, T. L. (1977). *Ibid.*, **43**, 173–179.

THORNBURG, J. E. & MOORE, K. E. (1973). *Neuropharmac.*, **12**, 853–866.

TRULSON, M. E., EUBANKS, E. E. & JACOBS, B. J. (1976). *J. Pharmac. exp. Ther.*, **198**, 23–32.