Effect of 5, 6-dihydroxytryptamine on the head twitches induced by 5-HTP, 5-HT, mescaline and fludiazepam in mice

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

Head twitches in mice might be induced by the increased activity of serotoninergic neurons (Corne, Pickering & Warner, 1963; Nakamura, Fukushima & Kitagawa, 1976; Nakamura & Fukushima, 1977a). Among drugs that induce such twitches are hallucinogens such as (+)-lysergic acid diethylamide and mescaline (Corne & Pickering, 1967; Silva & Calil, 1975), 5-hydroxytryptophan (5-HTP) (Corne & others, 1963), 5-hydroxytryptamine (5-HT) (Suchowsky, Pegrassi & others, 1969), and benzodiazepines (Nakamura & Fukushima, 1976b). But the detailed mechanism of the response is not yet known.

Baumgarten, Björklund & others (1971) reported that the intracerebral (i.c.) injection of 5,6-dihydroxytryptamine (5,6-DHT), a hydroxylated tryptamine derivative, caused a selective degeneration of the terminals of the central indolamine neurons and decreased the concentration of brain 5-HT. This paper reports the effect of the degeneration of serotoninergic neuron terminals, as a result of treatment with 5,6-DHT, on head twitches induced in mice by 5-HTP, 5-HT, mescaline and fludiazepam (ID-540), a new benzodiazepine possessing anticonvulsant, taming, sedative and muscle-relaxant properties in animals (Asami, Otsaka & others, 1974).

Ten male dd strain mice, 20–22 g, were used in each group. All experiments were in a room with a 12 h daylight cycle and a relatively constant environment (24 ± 1° and 55 ± 5% humidity). 5,6-DHT creatinine sulphate (50 μg free base), dissolved in 20 μl of isotonic saline (with 0·1% ascorbic acid) at 4°, was injected intracerebrally (Nakamura & Fukushima, 1976a) within 10 s under light ether anaesthesia. Vehicle-injected animals served as controls. On the 12th day after the injection of 5,6-DHT, the animals were tested by the procedure described below. Numbers of head twitches were counted for 2 min in white light, after the mice had been transferred singly to a plastic box (12 cm long × 18 cm high × 12 cm wide). The concentrations of 5-HT (Curzon & Green, 1971), dopamine and noradrenaline (Weil-Malherbe, 1971) in the brain were determined in aliquots of three pooled brains.

The concentrations of 5-HT, dopamine and noradrenaline in the brain were 0·69 ± 0·03, 1·17 ± 0·01 and 0·48 ± 0·03 μg g^{-1} brain, respectively for the animals injected with vehicle, and 0·49 ± 0·01, 1·14 ± 0·03 and 0·50 ± 0·03 μg g^{-1} brain, respectively for those injected with 5,6-DHT. Intracerebral injection of 5,6-DHT caused a significant reduction about 70% in the concentration of 5-HT in the brain of 5,6-DHT-treated mice compared with that in control mice. The concentrations of noradrenaline and dopamine in 5,6-DHT-treated mice were not significantly different from those of the controls.

The number of head twitches in control mice was 0·6 ± 0·3 for 2 min. A large dose of 5-HTP (90 mg kg^{-1}, i.v.) in control mice induced head twitches, but 40 mg kg^{-1} of 5-HTP did not significantly induce twitches. Intracerebral injection of 5-HT (10 and 20 μg) also induced head twitches that reached a maximum number at 8 min. Twitches induced by mescaline (12·5 mg kg^{-1}, i.v.) or fludiazepam (30 mg kg^{-1}, oral) reached a maximum number respectively at 4 and at 45 min after administration. The number of head twitches in 5,6-DHT-treated mice (0·6 ± 0·4) did not differ from that in control mice. When 5-HTP (40 mg kg^{-1}, i.v.) was injected into 5,6-DHT-treated mice, the number of twitches increased from 1·4 ± 0·5 to 33·2 ± 5·2 at 12 min after injection (Fig. 1A). The number of twitches induced by 5-HT (i.c.) was also markedly potentiated by pretreatment with 5,6-DHT, and reached a maximum (38·6 ± 7·1) at 8 min (Fig. 1B). The number of twitches induced by mescaline (12·5 mg kg^{-1},i.v.) in 5,6-DHT-treated mice significantly increased from 3·3 ± 0·6 to 8·1 ± 1·5 at 4 min (Fig. 1C). In contrast, fludiazepam at 30 mg kg^{-1} given to 5,6-DHT-treated mice produced a significant decrease in twitches (Fig. 1D).

Peripherally administered 5-HTP, a precursor of 5-HT, has been shown to increase the amount of 5-HT in the brain (Corrodi, Fuxe & Hökfelt, 1967). The head twitches induced by 5-HTP were proportional to the amount of 5-HT in the brain (Corne & others, 1963; Nakamura, Fukushima & Kitagawa, 1976). Intracerebral injection of 5-HT also induced head twitches in mice. The increase in twitch response to 5-HTP and 5-HT (i.c.) in the 5,6-DHT-treated mice could be explained, at least in part, by a presynaptic mechanism. The degeneration of 5-HT nerve terminals caused by 5,6-DHT treatment is paralleled by a decrease in the number of 5-HT reuptake sites. This would increase the free concentration of 5-HT, injected intracerebrally or formed from 5-HTP, to interact with the receptors. However, such a mechanism would not explain the potentiating effect of 5,6-DHT on twitches induced by mescaline which is a weak inhibitor of 5-HT uptake (Ross & Renyi, 1967) and would be likely to induce twitches as a result of its direct action on 5-HT receptors (Corne & Pickering, 1967; Nakamura & Fukushima, 1977a).

Administration of 6-hydroxydopamine (6-OHDA) has been shown to result in the destruction of catecholamine nerve terminals (Ungerstedt, 1971). Moreover, it has been recognized from biochemical and

* Correspondence.

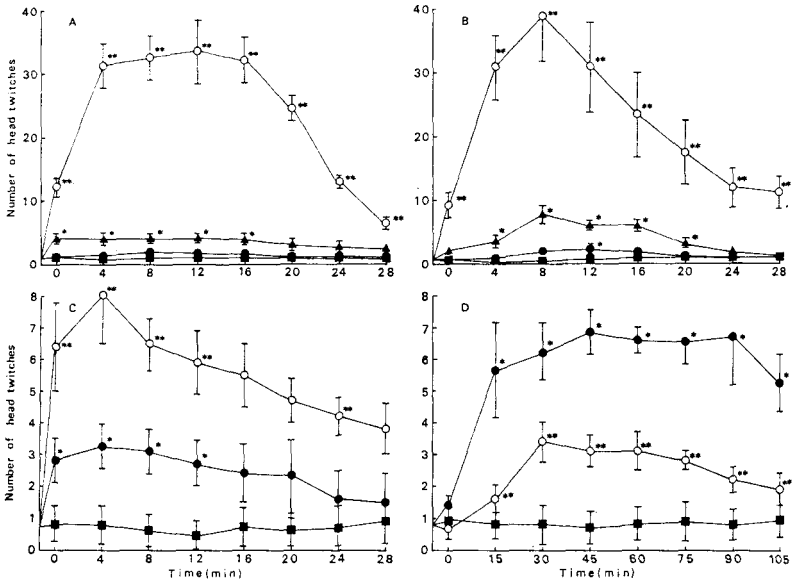

Fig. 1. The head twitches induced by 5-HTP, 5-HT, mescaline and fludiazepam in 5,6-DHT-treated mice. Animals received intracerebral injection of 20 μl of vehicle (closed circles, triangles and squares) or 50 μg of 5,6-DHT (open circles). On the 12th day after the injection of 5,6-DHT, the animals were tested. Numbers of head twitches were counted for 2 min at various times after the administrations of A—5-HTP (i.v.), ●,○ 40 mg kg^{-1}, ▲ 90 mg kg^{-1}. B—5-HT (i.c.) ●,○ 10 μg, ▲ 20 μg. C—mescaline (i.v.), ●,○ 12·5 mg kg^{-1}. D—fludiazepam (oral), ●,○ 30 mg kg^{-1}. ■—Saline for all parts. The number of head twitches was represented as mean ± s.e. from 10 mice. * differs from saline, $P < 0.05$; ** differs from vehicle, $P < 0.05$ (t-test). Ordinate: Number of head twitches. Abscissa: Time (min).

behavioural studies that the receptors in the dopaminergic systems become sensitive after the treatment with 6-OHDA (Iversen & Creese, 1975). A similar supersensitivity of 5-HT receptors might occur after 5,6-DHT (i.c.) in mice as suggested by Nygren, Fuxe & others (1974) who showed that the degeneration of spinal 5-HT nerve terminals induced by 5,6-DHT caused 5-HT receptor supersensitivity in rats. Such a mechanism would explain the enhanced response of head twitches with mescaline in 5,6-DHT-treated mice.

We previously reported that some benzodiazepines induced head twitches in mice, and had no influence on 5-HT uptake and release (Nakamura & Fukushima, 1976b, 1977a, b). Fludiazepam, one of the benzodiazepines, induced head twitches in control mice, but the twitches were reduced by pretreatment with 5,6-DHT. This shows that the mechanism of head twitches induced by fludiazepam differs from those of 5-HTP, 5-HT (i.c.) and mescaline. Moreover, it is consistent with the hypothesis that the head twitches induced by fludiazepam are caused by the drug's ability to activate the 5-HT receptors in the brain. The decrease in the head twitches induced by fludiazepam in 5,6-DHT-treated mice may demonstrate that the activating effect of fludiazepam on the 5-HT receptors in the brain is masked in 5,6-DHT-treated mice in which receptors in serotoninergic systems are supersensitive.

The results presented here show that although the head twitches induced by 5-HTP, 5-HT (i.c.), mescaline and fludiazepam are thought to be caused by the increased activity of serotoninergic neurons, their mechanism is not the same; 5-HTP and 5-HT (i.c.) induce twitches by increasing the free concentration of 5-HT at its receptor sites, mescaline by its direct action on 5-HT receptors, and fludiazepam by the activation of 5-HT receptors.

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Role of peripheral vascular resistance and reactivity in the interaction between clonidine and imipramine in spontaneously hypertensive rats

N. K. DADKAR*, A. N. DOHADWALLA, B. K. BHATTACHARYA, *Department of Pharmacology, Research Centre, Hoechst Pharmaceuticals Ltd, Mulund, Bombay 400 080, India*

Briant, Reid & Dollery (1973) reported that the hypotensive action of clonidine in patients is antagonized by simultaneous administration of tricyclic antidepressants. Although this interaction has been demonstrated in experimental hypertensive animals (Finch, Buckingham & others, 1975; Kaul & Grewal, 1975; Aroskar, Bhattacharya & others, 1976), the exact mechanism of this interaction is not clear. Van Zwieten (1975) has shown that tricyclic antidepressants administered via the vertebral artery reduces the hypotensive action of clonidine and proposed that the antagonism occurs at the level of α-receptor in the brain. However, a peripheral mechanism of action cannot be excluded completely since tricyclic antidepressants administered into the vertebral artery might have overflowed into the systemic circulation leading to a peripheral interaction with clonidine. According to Aström (1970) noradrenaline-induced hypertension is potentiated by tricyclic antidepressants indicating that these agents may have peripheral effects at postganglionic levels. In view of these findings, studies were carried out to investigate the action of imipramine on the peripheral vascular bed by perfusing the vascularly isolated but neurologically intact hindquarter and mesenteric artery preparations in spontaneously hypertensive (SH) rats.

The male SH rats (225–250 g) used were direct descendants of the original strain developed by Okamoto & Aoki (1963). Animals were anaesthetized with a combination of sodium pentobarbitone (20 mg kg^{-1}, i.p.) and urethane (500 mg kg^{-1}, i.p.). The hindquarter was perfused at a constant flow as described by Bhattacharya, Dadkar & Dohadwalla (1977). Blood from proximal part of the abdominal aorta was forced by a peristaltic pump (Desaga) into the distal part of the aorta.

The systemic blood pressure and perfusion pressure were measured with Statham P23Db pressure transducers and recorded on a physiological recorder (Hellige). The pump speed was so adjusted that the perfusion pressure and the systemic blood pressure were almost the same. Intra-arterial (i.a.) injections were made into the tubing towards the periphery. Heparin was injected (10 mg kg^{-1}) intravenously before cannulating the aorta.

The general technique for perfusing the mesenteric artery preparation was similar to that described for the hindquarter preparation. Blood from the carotid artery was forced by a peristaltic pump into the superior mesenteric artery.

Imipramine (0·05–0·3 mg, i.a.) adminstered into the hindquarter elicited a dose-related rise in perfusion pressure. With 0·3 mg it produced a sustained rise in

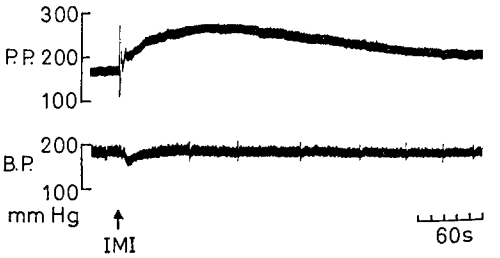

FIG. 1. Effect of intra-arterially administered imipramine (IMI 0·3 mg, i.a.) on blood pressure (B.P.) and perfusion pressure (P.P.) in perfused hindquarter preparation.

* Correspondence.