Duration of the effects of α-ethyl-4-methyl-*m*-tyramine, (H75/12) on brain 5-hydroxyindole concentrations in rats

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α-Ethyl-4-methyl-*m*-tyramine (H75/12), like 4-chloroamphetamine, is a lipophilic derivative of amphetamine with the ability to lower brain 5-hydroxytryptamine concentrations in rats and mice (Carlsson, Corrodi & others, 1969; Carlsson, Lindquist & others, 1970). An outstanding feature of 4-chloroamphetamine's depletion of 5-HT is the duration; brain 5-HT concentrations remain below normal for several months after a single dose (Sanders-Bush, Bushing & Sulser, 1972; Fuller & Snoddy, 1974).

H75/12 is like 4-chloroamphetamine in that (a) it is a lipophilic derivative of amphetamine, (b) it lowers brain 5-HT concentrations, (c) its lowering of 5-HT is blocked by inhibitors of amine uptake such as the tricyclic antidepressant drugs (Carlsson & others 1969; Dubnick, Rucki & Salama, 1973; Fuller, Perry & Molloy, 1975), and (d) it is not a substrate for monoamine oxidase. Meisch & Waldeck (1975) have reported that 5-HT concentrations in rat brain are depressed for as long as 8 h after a 25 mg kg^{-1} (i.p.) dose of H75/12, but the total duration of H75/12 action appears not to have been reported. We have assessed (a) the duration of action of H75/12 in rats, (b) how 5-hydroxyindoleacetic acid (5-HIAA) concentrations change after H75/12, and (c) whether H75/12 leads to reduced tryptophan hydroxylase activity as does 4-chloroamphetamine (Sanders-Bush & others, 1972).

Male albino Wistar rats, about 150 g, were given an injection of H75/12 (70 mg kg^{-1}, i.p.), and groups of 5 rats were decapitated at timed intervals thereafter. 5-Hydroxyindole concentrations in whole brain were measured spectrofluorometrically after condensation with *o*-phthalaldehyde (Miller, Cox & others, 1970).

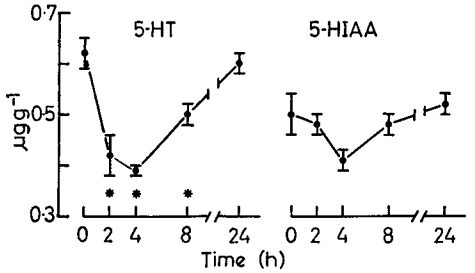

FIG. 1. Brain 5-HT and 5-HIAA concentrations ($\mu g\ g^{-1}$) after H75/12 injection (70 mg kg^{-1}, i.p.) into rats. Mean values ± standard errors for 5 rats per group are shown. * indicates time at which 5-hydroxyindole concentrations were significantly different from the zero time values ($P<0.05$).

* Correspondence.

Tryptophan hydroxylase activity was measured by the spectrofluorometric method of Gal & Patterson (1973).

Fig. 1 shows brain 5-HT and 5-HIAA concentrations after the injection of H75/12. 5-HT concentrations were significantly reduced at 2, 4, and 8 h after drug injection but had returned to control values within 24 h. 5-HIAA concentrations, although lower than controls, were not significantly different at any time. Fig. 2 shows the results of an experiment in which tryptophan hydroxylase in addition to 5-HT and 5-HIAA concentrations were measured 4 h after drug injection. Here there was a significant reduction in all three parameters.

The decrease in 5-HIAA concentrations was also observed by Dubnick & others (1973) after H75/12 administration to mice. Although H75/12 has been suggested to deplete 5-HT by releasing it from storage granules (Carlsson & others, 1970), the decrease in 5-HIAA indicates that H75/12 has an action different from that of a typical releasing agent like reserpine, which elevates 5-HIAA concentrations because the released 5-HT becomes susceptible to attack by monoamine oxidase. In this, H75/12 resembles 4-chloroamphetamine, which lowers 5-HIAA as well as 5-HT concentrations. The fall in 5-HIAA concentrations effected by 4-chloroamphetamine has been attributed either to inhibition of monoamine oxidase or tryptophan hydroxylation rather than to release of 5-HT as the predominant factor in the 5-HT depletion.

The duration of 5-HT depletion by H75/12 is very short compared with that produced by 4-chloro-

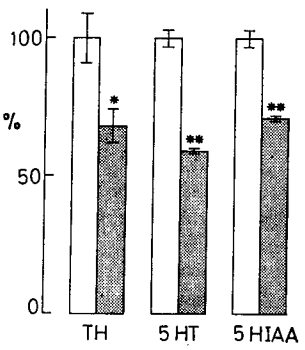

FIG. 2. Brain tryptophan hydroxylase (TH), 5-HT and 5-HIAA concentrations at 4 h after the intraperitoneal injection of saline (open bars) or H75/12 (70 mg kg^{-1}, i.p.) (shaded bars) into rats. Mean values ± standard errors for 5 rats per group are presented as percentage of the control mean which was 3·83 ± 0·36 $\mu g\ g^{-1}\ h^{-1}$ for tryptophan hydroxylase, 0·60 ± 0·02 $\mu g\ g^{-1}$ for 5-HT, and 0·39 ± 0·01 $\mu g\ g^{-1}$ for 5-HIAA. * indicates significant drug effect at $P<.025$, and ** indicates difference at $P<.001$.

amphetamine, despite the fact that, like 4-chloroamphetamine (Meek, Fuxe & Carlsson, 1971) it is apparently a substrate for the amine pump on the neuronal membrane. This suggests that the ability to be taken into the neuron by the amine pump is not sufficient to account for the long duration of action of 4-chloroamphetamine.

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Effect of synthetic motilin and related polypeptides on contraction of gastrointestinal smooth muscle

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Brown, Johnson & Magee (1966) found that when the duodenum of the dog was made alkaline with tris buffer (pH 9·0), powerful motor activity was observed in transplanted pouches of the fundic gland area of the stomach. The observation caused these authors to suggest that the increased motor activity could be due to the release of a stimulatory substance from duodenal mucosa. This hypothesis was seen supported by their experimental results that intravenous injection of duodenal extract containing pancreozymin-cholecystokinin produced a similar increase in motor activity in fundic pouches (Brown & Parkes, 1967).

This substance, named motilin, is a docosapeptide which is distinct from other gastrointestinal hormones extracted from the same area of the small intestine. The entire amino-acid sequence of porcine motilin was determined by Schubert & Brown (1974), after a minor correction of their previous formula (Brown, Cook & Dryburgh, 1973).

Yajima, Kai & Kawatani (1975) have synthesized the peptide corresponding to the newly revised sequence of motilin by a method different from that employed by Wünsch, Brown & others (1973) who synthesized [13-Nle, 14-Glu]-motilin. 13-Nle-motilin has been shown to have a contraction-promoting effect—mainly on the smooth muscle of the gastric antrum, the duodenum and the colon in the rabbit and man, but little or no effect on other parts of the intestine (Domschke, Strunz & others, 1974). However, no studies have so far investigated the effect of synthetic motilin on contraction of gastrointestinal smooth muscle. Besides motilin, the docosapeptide, Yajima & others (1975) have synthesized three shorter chain peptides related to motilin whose amino-acid sequences are shown in Table 1.

The aim of the present experiment was to investigate the influences of synthetic motilin and related polypeptides on contraction of gastrointestinal smooth muscle and to clarify the amino-acid sequence necessary for the effect.

The smooth muscle strips (Table 2) were suspended in a 20 ml organ bath containing Tyrode solution gassed with air and maintained at 30 ± 2°. For stomach, a strip of longitudinal muscle approximately 0·5 cm wide and 2 cm in length was removed along

Table 1. *Amino-acid sequences of synthetic motilin and related polypeptides.*

Motilin	H-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH
M[6–22]	H-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH
M[9–22]	H-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH
M[14–22]	H-Gln-Glu-Lys-Glu-Arg-Asn-Lys-Gly-Gln-OH

† Correspondence.