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Antagonism by (−)-methadone of the depletion of 5-hydroxytryptamine in rat brain by *p*-chloroamphetamine

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Ciofalo (1974) reported methadone to be a potent inhibitor of the neuronal uptake of 5-hydroxytryptamine (5-HT) in vitro. Some uncertainty has arisen and persisted about the ability of methadone to inhibit 5-HT uptake in vivo. Attempts to demonstrate reduced uptake of 5-HT in vitro by preparations from brains of rats treated in vivo with methadone at analgesic doses have generally failed (Moffat & Jhamandas 1976; Slotkin et al 1978; Donzanti & Warwick 1979), though such inhibition was observed in one study after a high, toxic dose of methadone (Moffat & Jhamandas 1976). Methadone was reported not to alter 5-hydroxyindoleacetic acid (5-HIAA) concentration or turnover in rat brain (Goodlet & Sugrue 1974; Fuller & Perry 1976; Miranda et al 1979) or mouse brain (Fuller & Perry 1976), whereas inhibition of 5-HT uptake is known to decrease 5-HIAA concentration owing to a decrease in 5-HT turnover. Also, methadone was reported not to antagonize the depletion of rat brain 5-HT by *p*-chloroamphetamine (PCA) (Fuller & Perry 1976) or fenfluramine (Miranda et al 1979), two depleting agents whose effects are antagonized by inhibition of uptake into 5-HT neurons. Thus in vivo studies have generally agreed that methadone does not inhibit 5-HT uptake at doses known to be effective in producing analgesia.

Recently Ahtee & Carlsson (1979) suggested that methadone failed to lower 5-HIAA concentration in brain because an opiate receptor-mediated increase in 5-HT turnover counteracted the decrease in 5-HT turnover that normally would result from 5-HT uptake inhibition. According to their hypothesis, methadone has dual influences on 5-HIAA concentration: (1) an opiate action that tends to increase 5-HIAA and (2) 5-HT uptake inhibition that tends to decrease 5-HIAA, the net effect being no change. Ahtee & Carlsson (1979) showed that blocking the opiate action of methadone revealed its ability to lower 5-HIAA and interpreted that lowering as evidence that methadone inhibited 5-HT uptake in vivo.

We considered that the failure of methadone to antagonize PCA-induced depletion of brain 5-HT in our earlier experiments could have been due to a very short duration of action of methadone, since uptake into 5-HT neurons must be inhibited throughout the period between the time of PCA injection and the time at which rats are killed in order for 5-HT depletion to be antagonized (Fuller et al 1978). Additional experiments were undertaken and have indicated that methadone can antagonize PCA-induced depletion of brain 5-HT but has a short duration of action and requires doses higher than those needed for analgesia.

Male Wistar rats from Harlan Industries, Cumberland, Indiana, were used. (−)Methadone hydrochloride was synthesized at Eli Lilly and Company, and (±)*p*-chloroamphetamine hydrochloride was purchased from the Regis Chemical Company. The drugs were dissolved in pyrogen-free, glass-distilled water and injected i.p. in a volume of 1 ml kg^{-1}. Rats were decapitated, and forebrains (whole brain minus brain stem and cerebellum) were quickly removed, frozen on dry ice, and stored at −15 °C before analysis. 5-HT concentration was determined spectrofluorometrically after extraction and reaction with *o*-phthalaldehyde (Miller et al 1970) or in one experiment (data in Table 1) by high performance liquid chromatography with electrochemical detection. In the latter case, brains were homogenized in 0·1 M trichloroacetic acid. The homogenate was centrifuged, and an aliquot of the supernatant fluid was injected directly onto the reverse phase column (Dupont Zorbax C_8 column with a Rheodyne C_8 guard column). The electrochemical detector was set at 0·75 V. The rat tail jerk analgesic test was conducted in rats fasted overnight. The tail of the rat was held near a nichrome resistance wire through which a current (AC) of 6·5 A was passed to generate heat. When the rat jerked its tail a trained observer recorded the latency of the response. The tail was placed in the proximity of the hot wire for a period not exceeding 15 s.

Table 1 shows the ability of two successive doses of (−)-methadone to antagonize brain 5-HT depletion by

Table 1. Antagonism by two successive doses of (−)-methadone of the PCA-induced depletion of rat brain 5-HT concentration. *p*-chloroamphetamine hydrochloride (PCA) was injected i.p. 3 h before rats (170–200 g body weight) were killed. (−)-Methadone hydrochloride (5 mg kg^{-1}, i.p.) was injected 10 min before PCA and again 1·5 h after PCA injection. Mean values ± standard errors for 5 rats per group are shown.

Dose of PCA mg kg^{-1}	Brain 5-HT, ng g^{-1} Control	Methadone
0	448 ± 22	448 ± 17
5	165 ± 11* (−63%)	362 ± 38** (−19%)

* Significant depletion of 5-HT ($P < 0.01$).
** Significant antagonism of PCA effect ($P < 0.01$).

* Correspondence.

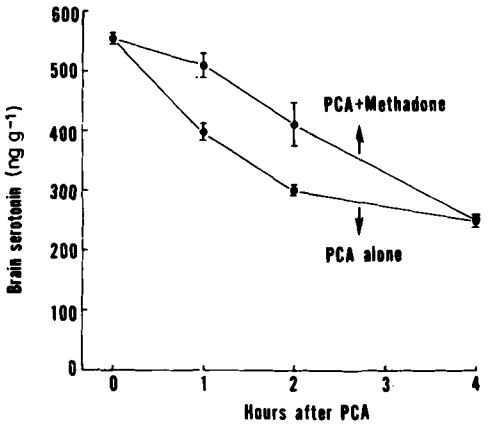

FIG. 1. 5-HT concentration in rat brain after injection of PCA alone or with (−)-methadone. Single doses of PCA and of (−)-methadone (each at 5 mg kg^{-1}, i.p.) were injected at zero time. Mean values ± standard errors for 5–10 rats (130–150 g body weight) per group are shown.

PCA in rats. (−)-Methadone alone had no significant effect on brain 5-HT concentration, but reduced the degree of depletion of brain 5-HT by PCA from 65 to 19%. The difference in 5-HT concentration in PCA-treated rats with and without (−)-methadone was highly significant ($P < 0.01$). Carlsson & Lindqvist (1969) had reported antagonism by methadone (presumably racemic) of cerebral 5-HT depletion by 4-methyl-*p*-ethyl-*m*-tyramine in mice; apparently two successive doses of methadone had been used in their experiment as well.

Fig. 1 shows the time course of the depletion of brain 5-HT by PCA. The depletion became progressively greater with time at 1, 2 and 4 h after PCA, statistically significant depletion occurring at all times points ($P < .05$). (−)-Methadone injected at the same time as PCA significantly antagonized the depletion of 5-HT at 1 h and at 2 h after PCA ($P < 0.05$). By 4 h after PCA, the depletion of 5-HT by PCA was identical with or without (−)-methadone injection.

Table 2 compares the analgesic effects and PCA-antagonizing effects of various doses of (−)-methadone at 1 h. The 0·5 mg kg^{-1} dose had no analgesic effect, but the 1 mg kg^{-1} dose significantly increased rat tail jerk latency and the 2 mg kg^{-1} dose produced essentially maximum effect. In contrast, no antagonism of PCA-induced depletion of brain 5-HT was found except at the 4 mg kg^{-1} dose of (−)-methadone, which was a supra-maximal analgesic dose. These data suggest that inhibition of 5-HT uptake is not involved in the analgesic action of methadone and that inhibition of uptake does not occur until overtly toxic doses in excess of those producing maximum analgesia are reached.

Table 2. Comparative analgesic effects and PCA-antagonizing effects of (−)-methadone in rats. Rats weighing 75–90 g were used. To demonstrate antagonism of PCA, (−)methadone hydrochloride was injected s.c. 5 min before *p*-chloroamphetamine hydrochloride (5 mg kg^{-1} i.p.). Rats were killed 1 h after. 5-HT concentration in control rats was 0·71 ± ·01 μg g^{-1}. 5-HT concentration was measured in rats treated with each dose of methadone without PCA and in no case differed significantly from control. Analgesic effects were measured following the s.c. administration of (−)-methadone.

Dose of methadone mg kg^{-1}	Brain 5-HT in PCA-treated rats, μg g^{-1} (mean ± s.e. n = 5)	Rat tail jerk latency (s) (mean ± s.e. n = 10)
0	0·56 ± 0·02	6·78 ± 0·14
0·5	0·60 ± 0·02	6·65 ± 0·18
1	0·61 ± 0·05	8·68 ± 0·63*
2	0·55 ± 0·03	14·96 ± 0·04*
4	0·71 ± 0·03*	15·00 ± 0·00*

* Significant effect of methadone ($P < 0.01$).

The short duration of protection by (−)-methadone against brain 5-HT depletion explains why we failed to observe protection by a single dose of methadone when we measured 5-HT 4 h after PCA (Fuller & Perry 1976) and may explain why Miranda et al (1979) failed to observe protection by a single dose of methadone when they measured 5-HT 2 h after fenfluramine injection (2·5 h after injection of methadone at 4 mg kg^{-1}).

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