The effect of pethidine on the 5-hydroxytryptamine and 5-hydroxyindoleacetic acid content of the mouse brain

Morphine decreases hypothalamic noradrenaline in cats (Vogt, 1954) and dopamine in mouse brain (Takagi & Nakama, 1966). Chronically administered morphine is reported to increase the turnover of 5-hydroxytryptamine (5-HT) in the brain of rats and mice (Shen, Loh & Way, 1970; Haubrich & Blake, 1969; Bowers & Kleber, 1971) although not in all experiments (Marshall & Grahame-Smith, 1970; Algeri & Costa, 1971; Cheney, Goldstein & others, 1971). Bowers & Kleber (1971) showed that methadone increases mouse brain 5-hydroxyindoleacetic acid (5-HIAA) content both in acute and chronic experiments. However, in heroin addicts treated with methadone, probenecid caused less increase in the 5-HIAA content of cerebrospinal fluid than in control patients (Tamarkin, Goodwin & Axelrod, 1970).

We now report the effect of pethidine on the 5-HT and 5-HIAA content of the mouse brain *in vivo*. Probenecid blocks the active transport of 5-HIAA in rat and mouse brain (Neff, Tozer & Brodie, 1967; Sharman, 1969). Chronic treatment with lithium interferes with the monoamine uptake by synaptosomes and platelets (Colburn, Goodwin & others, 1967; Murphy, Colburn & others, 1969).

White male mice, 20–25 g, were kept in groups of eight; experiments were made between 4 and 8 p.m., at 25°. Drugs were injected in 0·1 ml/10 g and controls injected with similar amounts of saline. The animals were decapitated always between 7 and 8 p.m. and the brains, except cerebellum, were rapidly removed, weighed and stored at −20° for less than 5 days. 5-HT and 5-HIAA were estimated in the same tissue sample spectrophotofluorimetrically (Ahtee, Sharman & Vogt, 1970). Pethidine did not interfere with the fluorescence of 5-HT or 5-HIAA. Pethidine hydrochloride was dissolved in saline. Probenecid (Benemid; a gift from Merck Sharp & Dohme Ltd) was dissolved in a minimum volume of 1N NaOH and the pH of the solution was adjusted to 7–8 with 0·1N HCl and diluted further with saline. The doses are expressed as base. In one series of experiments mice were pretreated with lithium chloride administered in drinking water (200 mg LiCl in 1000 ml of tap water) for three months.

Pethidine alone (80 mg/kg, i.p., 2 h) did not alter the concentration of brain 5-HT or 5-HIAA (Table 1). In preliminary experiments, 30, 60 and 120 mg/kg of pethidine were given intraperitoneally to the mice. No changes in brain 5-HT or 5-HIAA content were observed 2 h after the lower doses and 30 min after the highest dose. In our mouse strain the LD50 of pethidine is 113 mg/kg, i.p. (Jounela, Saarnivaara & Ahtee, 1971).

Probenecid (200 mg/kg, i.p., $1\frac{3}{4}$ h) increased the 5-HIAA content in the brain of the saline- and pethidine-treated mice. But, the increase in the brain 5-HIAA content was statistically significantly higher in the control group ($P < 0.001$ by *t*-test after pairing values). The time course of the effect of combined pethidine-probenecid treatment on the 5-HIAA content was also studied by giving probenecid

Table 1. *Effect of pethidine* (80 mg/kg, i.p., 2 h) *and probenecid* (200 mg/kg, i.p., $1\frac{3}{4}$ h) *on the 5-HT and 5-HIAA content of mouse brain.* Means ± s.e.; number of mice in brackets.

Treatment	5-HT μg/g	5-HIAA μg/g
Saline	0·577 ± 0·033 (8)	0·367 ± 0·022 (7)
Probenecid	0·609 ± 0·033 (10)	0·648 ± 0·062 (7)
Pethidine	0·619 ± 0·017 (7)	0·343 ± 0·028 (7)
Pethidine + probenecid	0·623 ± 0·017 (9)	0·494 ± 0·020 (7)

1¾ h before killing and varying the duration of pethidine treatment. The lowest 5-HIAA content was found 2 h after administration of pethidine; 3 h after pethidine the 5-HIAA values were 0·556 and 0·563 μg/g and 4 h after pethidine 0·589 ± 0·066 μg/g (mean ± s.e.; n = 4). Lithium pretreatment neither altered the brain 5-HT and 5-HIAA contents nor modified the effects of pethidine or probenecid. In the lithium-pretreated mice the increase in the brain 5-HIAA content was twice as much after probenecid only, as after the combination of pethidine and probenecid.

Fifteen min after injection of pethidine (80 mg/kg, i.p.) the mice became excited, jumpy, had motor disturbances and exhibited the Straub tail phenomenon. These symptoms wore off in about 2 h. At this time the mice were sedated. Probenecid or lithium did not modify these symptoms. No, or only slight, decrease of rectal temperature was caused by pethidine or probenecid at the temperature of 25°.

Thus, pethidine decreases the probenecid-induced accumulation of 5-HIAA in mouse brain, an effect which could arise from the inhibition of the re-uptake of 5-HT into neurons, whereby 5-HT would not reach intraneuronal monoamine oxidase (MAO). This possibility is supported by the finding that at 50 mg/kg, i.p., pethidine prevents the 4-methyl-α-ethyl-*m*-tyramine (H 75/12)-induced 5-HT displacement in the mouse brain *in vivo* (Carlsson & Lindqvist, 1969) as well as by our experiments in which pethidine prevented the uptake of 5-HT into human blood platelets *in vitro* (Ahtee & Saarnivaara, 1970a, b). Furthermore, Meek & Werdinius (1970) have shown that chlorimipramine, a potent inhibitor of 5-HT uptake, decreases the probenecid-induced accumulation of 5-HIAA in rat brain. But there are several other possible causes for the effect of pethidine—such as the inhibition of 5-HT oxidation by MAO, decrease in the synthesis or release of 5-HT, and pethidine might in one way or another antagonize the blockade of active transport caused by probenecid.

At least some actions of pethidine could be mediated by alterations of the brain 5-HT content. Recent evidence shows that the toxic reactions of pethidine when combined with MAO inhibitors are at least partially due to increased 5-HT concentrations in the mouse brain (Jounela, 1970; Rogers & Thornton, 1969; Rogers, 1971). Moreover, 5-HT is involved in the perception of pain and in the mechanism of action of several analgesics (Tenen, 1967 & 1968).

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Comparison of four vehicles for intraperitoneal administration of Δ^1-tetrahydrocannabinol*

Recently there has been much interest in research on Δ^1-tetrahydrocannabinol, commonly designated by the abbreviation Δ^1-THC (or Δ^9-THC), which is believed to be the principal active constituent of marihuana. However, a practical difficulty of research with Δ^1-THC is its insolubility in water and many other common solvents. It is soluble in ethanol, but this vehicle is pharmacologically active, in particular resembling effects of Δ^1-THC in general depressant action (Kubena & Barry, 1970) and in stimulation of the adrenal-pituitary system (Kubena, Perhach & Barry, 1971). Another solvent for Δ^1-THC, propylene glycol, was used by Bose, Saifi & Bhagwat (1964) and by Bicher & Mechoulam (1968), but this is also a general depressant when given in large amounts (Bost & Ruckebusch, 1962). Therefore, in the studies cited and others (Sofia & Barry, 1970; Sofia, Dixit & Barry, 1971), the present authors used a suspension in 10% propylene glycol, 1% polysorbate (Tween) 80 and isotonic saline to minimize the amount of propylene glycol administered.

Fenimore & Loy (1971) have recently suggested the use of a suspension of Δ^1-THC in polyvinylpyrrolidone (PVP), a plasma expander. Other vehicles used for intraperitoneal administration of Δ^1-THC include a suspension in bovine serum albumin (Dewey, Peng & Harris, 1970; McMillan, Harris & others, 1970) and a suspension in Tween 80-saline (Holtzman, Lovell & others, 1969). Recently, Ho, Fritchie & others (1971) presented evidence for poor absorption of Δ^1-THC after intraperitoneal injection in a Tween-80-saline suspension. They recommended that the drug be administered intravenously, but this route has the drawback of being more difficult and stressful, especially for rats, and not directly comparable with data on other drugs, which are mostly administered intraperitoneally.

The present report compares four vehicles in efficacy and duration of effect after intraperitoneal injection of Δ^1-THC. The latency to convulsion in mice following a maximal electroconvulsive shock (ECS) was used as a bioassay of the Δ^1-THC effect. Recent work (Sofia, Solomon & Barry, 1971) has shown this to be a sensitive measure of Δ^1-THC, even at a low dose, when injected intraperitoneally in a 10% propylene glycol–1% Tween 80-saline suspension.

The experiment was made on 384 male albino mice (Swiss-Webster), 20 to 22 g (Hilltop Lab Animals, Inc., Scottdale, Pa.). They were divided randomly among the

*Numbered Δ^9 according to IUPAC rules.