Pethidine interaction with clorgyline, pargyline, or 5-hydroxytryptophan: lack of enhanced pethidine lethality or hyperpyrexia in mice

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Pethidine causes excitation and a fatal hyperpyrexia in rabbits pretreated with a monoamine oxidase inhibitor (MAOI) (Penn & Rogers, 1971 & refs cited). Likewise in mice, tranylcypromine-pethidine interaction produces a fatal hyperpyrexia (Rogers & Thornton, 1969). Pethidine's effect to block the reuptake of 5-hydroxytryptamine (5-HT) (Fuller & Snoddy, 1975 & refs cited) has been suggested to account for its greater toxicity when administered to animals pretreated with MAOIs (Penn & Rogers, 1971; Gessner & Soble, 1973). However, Fuller & Snoddy (1975) reported reduced lethality of pethidine after pretreatment with fluoxetine, a potent and specific inhibitor of 5-HT reuptake (Wong, Bymaster & others, 1975). The results of Fuller & Snoddy suggested that the increased lethality associated with pethidine-MAOI interaction is not necessarily due to the combination of blocked serotoninergic reuptake and MAO inhibition resulting in increased brain 5-HT concentrations.

In mice, the enhanced lethality of pethidine has been observed only with tranylcypromine and iproniazid (Rogers & Thornton, 1969; Gessner & Soble, 1973; Fuller & Snoddy, 1975), two relatively non-specific MAOIs (cf. Fuentes & Neff, 1975). We decided to investigate whether hyperpyrexia and increased lethality with pethidine could be produced with pretreatments of the more specific MAOIs, clorgyline and pargyline, and with treatment with L-5-hydroxytryptophan, the immediate precursor of 5-HT. Since the interaction of pethidine and MAOIs in man and rabbit is characterized by hyperpyrexia, the body temperatures of these mice were monitored twice after injection of pethidine.

Dublin Albino male mice, 22–32 g were isolated into groups of five and maintained at 25°. The MAO inhibitors used were tranylcypromine sulphate (Smith, Kline & French), administered 4 h before pethidine hydrochloride (Sterling-Winthrop); pargyline hydrochloride (Abbott Laboratories), 50 mg kg^{-1}, 2 h before pethidine; and clorgyline (May & Baker), 20 mg kg^{-1}, 2 h before pethidine. As an alternative method of producing increased brain 5-HT, 200 mg kg^{-1} of L-5-hydroxytryptophan (5-HTP) (Sigma) was injected simultaneously with pethidine. These doses and pretreatment times were selected because they have been reported to produce an approximate doubling of brain 5-HT concentrations (Tozer, Neff & Brodie, 1966; Rogers & Thornton, 1969; Yang & Neff, 1974; Tabakoff & Moses, 1976) in mice and rats. Control animals were injected with distilled water 4 h before pethidine. Doses were dissolved in distilled water and were given intraperitoneally in a volume of 1 ml/100 g weight. Rectal temperatues at 0, 45, and 90 min after pethidine were measured by a Yellow Springs thermistor probe inserted 2 cm into the rectum.

Fig. 1 shows the effects of pethidine injected in control mice receiving only water pretreatment. Doses of 150 and 125 mg kg^{-1} of pethidine produced 80 and 40% lethalities, respectively, establishing an estimated LD 50 of 138 mg kg^{-1}, whereas doses of 100 and 75 mg kg^{-1} produced no fatal reactions. Also, a marked decrease in rectal temperatures accompanied the pethidine injection after the 150, 125, and 100 mg kg^{-1} dosages. Other symptoms noted included motor restlessness, hyperexcitability, spastic tremors, and the Straub-tail reflex.

As with previous studies (Gessner, Clarke & Adler, 1974; Fuller & Snoddy, 1975 & refs cited), upon pethidine administration, tranylcypromine-pretreated mice showed a greater lethality at all doses than did the control group (estimated LD50 of 62 mg kg^{-1}). Moreover, the pretreatment with tranylcypromine led to a condition of pethidine-induced hyperpyrexia. In contrast to this, pretreatment with pargyline and clorgyline did not produce these effects (Fig. 2) in that the MAOI pretreatment consistently resulted in a pethidine-induced temperature decrease, as was found with the water-

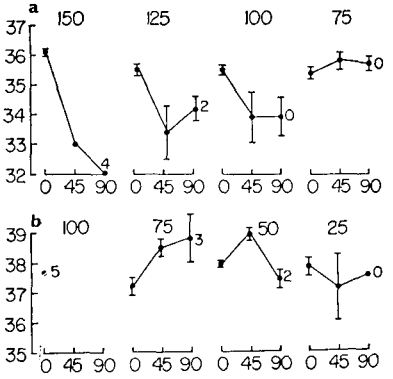

FIG. 1. Effects of pethidine on the rectal temperature (°C; ordinate) of mice pretreated 4 h before with a: water or b: tranylcypromine. Doses of pethidine (mg kg^{-1}) are given at the top (150, 125, 100, etc.). Rectal temperatures were measured at the time of pethidine administration (0 min) and at 45 and 90 min after pethidine. Numbers beside the recorded temperatures indicate the number of mice that died out of a possible 5. Absicssa: Time (min) after pethidine injection.

* Correspondence.

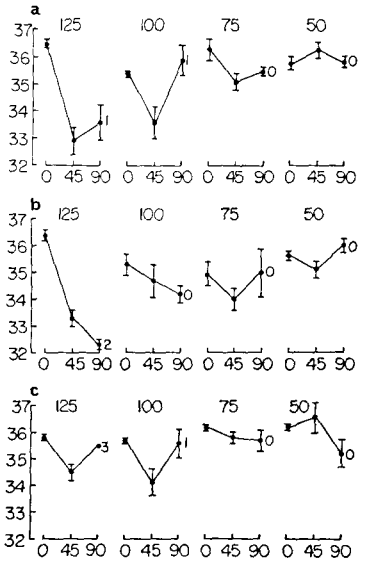

FIG. 2. Effects of pethidine on the rectal temperature (°C; ordinate) of mice pretreated 2 h previously with a: clorgyline or b: pargyline, or c: simultaneously with L-5-hydroxytryptophan. Details are as in Fig. 1.

pretreated control animals, and there was no incidence of enhanced pethidine lethality.

Furthermore, pretreatment with 5-HTP, had little effect in interaction with pethidine (Fig. 2). Lethality was only slightly increased compared with control mice, and the pethidine-induced temperature changes appeared to demonstrate only hypothermia. These results run counter to the usual explanation of the MAOI pethidine interaction; MAO inhibition increases brain 5-HT; this results in more 5-HT being released into the synaptic cleft, and pethidine blocks the reuptake of 5-HT resulting in signs of toxicity.

This explanation has been supported by studies in rabbits (Gong & Rogers, 1973; Osman & Eltayeb, 1977 & refs cited) which show that depletion of brain 5-HT or pretreatment with 5-HT receptor antagonists will reduce or prevent this toxic interaction, whereas drugs affecting other neurotransmitter systems are ineffective. This conclusion in rabbits is supported also by the fact that the effect occurs only with narcotic-related drugs which block the reuptake of 5-HT, such as pethidine and dextromethorphan, and not with narcotics which do not block 5-HT reuptake such as morphine and pentazocine (Penn & Rogers, 1971; Sinclair, 1972). Likewise the hyperthermic response of rabbits to 5-HTP is potentiated by pethidine.

The present data, and previous reports, indicate that the increased lethality of pethidine in the tranylcypromine-pretreated mouse, however, is not due simply to an MAOI-induced increase in brain 5-HT in combination with blockade of the reuptake of 5-HT. The first evidence for this conclusion is that in the tranylcypromine-pretreated mouse, there is enhanced lethality also with morphine, pentazocine, and phenazocine (Rogers & Thornton, 1969), narcotics which do not block the reuptake of 5-HT. The second evidence comes from the present study which shows that there is not enhanced lethality in mice pretreated with clorgyline or pargyline nor is there enhanced lethality from pethidine in mice treated with a large dose of 5-HTP. The third evidence for the above conclusion comes from the findings of Fuller & Snoddy (1975). They showed that in tranylcypromine-pretreated mice, serotoninergic reuptake blockade by fluoxetine did not produce greater lethality than in animals not treated with tranylcypromine.

The fact that serotoninergic stimulation increases body temperature in rabbits (Weber & Angell, 1967; Jacob, Girault & Peindaries, 1972; Quock, Beal & Chan, 1976 & refs cited) but decreases body temperature in mice (Barofsky & Feldstein, 1970), leads us to speculate that the specific interaction between pethidine and MAOIs only occurs in animals in which serotoninergic stimulation leads to hyperpyrexia. Thus the specific interaction (seen with pethidine, but not morphine and pentazocine, and with all MAOIs, not just tranylcypromine) is observed in rabbits, but not in mice. This hypothesis is supported by recent experiments (Sinclair & Lo, 1977) showing that fluoxetine in phenelzine-treated rabbits produced a lethal hyperpyrexia. Thus fluoxetine produces hyperpyrexia and enhanced lethality when given to MAOI-treated rabbits (Sinclair & Lo, 1977), but not in MAOI-treated mice (Fuller & Snoddy, 1975). The present data also support the recent hypothesis by Jounela, Mattila & Knoll (1977) from studies in rabbits that the pethidine-MAOI interaction only occurs with inhibition of both the A and B forms of MAO using non-specific MAOIs such as tranylcypromine, phenelzine, or iproniazid, but does not occur if only the A form (by clorgyline) or B form (by pargyline) of MAO is inhibited.

Thus the recent results from the various studies on the pethidine-MAOI interaction suggest that the generality is not as great, and the mechanism is not as simple, as it was previously thought to be.

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Selectivity of bethanechol on muscarinic receptors

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Bethanechol is a cholinergic agonist with little if any nicotine actions (Goodman & Gilman, 1975; Martindale, 1977). Evidence for this selective action is based on the absence of any pressor activity of bethanechol in the atropinized cat (Simonart & Simonart, 1935) or dog (Farber, 1936). Although bethanechol is used to demonstrate the effects of muscarinic stimulation in the gastrointestinal tract its selectivity of action at this site has not been established. A suitable gastrointestinal preparation for demonstrating selective stimulation of muscarinic receptors is the isolated human colon. Circular and longitudinal muscle layers of the colon contain both muscarinic and nicotinic receptors, the former mediating contraction the latter relaxation (Fishlock & Parks, 1963; Bucknell & Whitney, 1964).

Circular muscle strips about 20 mm long and 2 mm wide were taken from specimens of colon removed at operation. Muscle strips prepared only from healthy tissue consisted of the full thickness of bowel wall with the mucosa removed. The strips were suspended in Krebs bicarbonate solution at 37° aerated with a mixture of 5% CO_2 in oxygen. Movements of the muscle were recorded by a Devices or Rikadenki recorder using an isotonic transducer (load 0·5–1 g). The Krebs bicarbonate solution contained (mM) Na 140, K 5·9, Ca 2·5, Mg 1·2, Cl 122, HCO_3 25, HPO_4 1·2, SO_4 1·2, dextrose 11·5. Circular colonic muscle strips usually lost tone before completion of the experiment, thus making it difficult to obtain relaxation to nicotine receptor stimulation. This was avoided by incubating muscle strips with barium chloride ($BaCl_2$) which maintained the tone of the strips at a higher level and made it possible for nicotine receptor stimulation to relax the muscle strip. One experiment was completed without addition of $BaCl_2$ (Fig. 1) and the results were similar to those experiments using $BaCl_2$.

Acetylcholine (0·2–7·1 × 10^{-6} M) and bethanechol (0·6–40 × 10^{-6} M) contracted circular colonic muscle strips, this effect was blocked by hyoscine (4·6 × 10^{-6} M). Increasing the concentration of acetylcholine (0·9–4·4 × 10^{-4} M) in the presence of hyoscine (4·6 × 10^{-6} M) caused a biphasic response (relaxation followed by contraction); the relaxation was blocked by hexamethonium (35·6 × 10^{-6} M, Fig. 1). In contrast when the concentration of bethanechol was increased in the

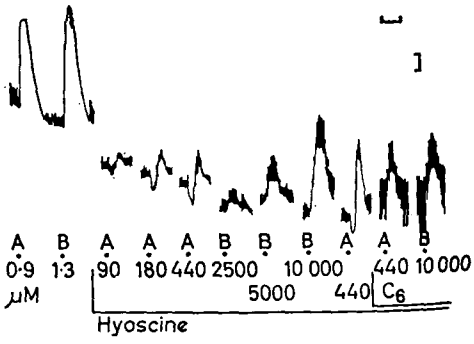

FIG. 1. Human circular sigmoid colon: showing responses to acetylcholine (A) and bethanechol (B) before and after hyoscine (4·6 × 10^{-6} M). Hexamethonium (C_6, 35·6 × 10^{-6} M) was then tested against bethanechol and the modified response to acetylcholine. A 2 min contact time was used for the agonists. Horizontal scale: 5 min; vertical scale: 1 cm.