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Interaction of selective inhibitors of monoamine oxidase with pethidine in rabbits

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Pethidine can cause serious reactions in patients treated with monoamine oxidase (MAO) inhibitors [1]. Similar toxic reactions can be produced to rabbits and aggregated mice, and biogenic amines may play the main role in its mechanism. Both 5-hydroxytryptamine (5HT) and noradrenaline (NA) have been suggested to be involved [2, 3]. We have provided indirect evidence to suggest that phenylethylamine (PEA), a metabolite of phenylalanine, and a substrate for MAO, could contribute to initiating the toxic interaction in rabbits [3].

Two forms of MAO have been demonstrated in the mammalian brain both *in vitro* and *in vivo* [4, 5]. 5HT and NA are the preferred substrates of MAO type A, being sensitive to selective inhibition by clorgyline. PEA is the preferred substrate of MAO type B which is sensitive to selective inhibition by deprenyl in the rat brain [6, 7] but not in the rabbit brain [8]. Phenelzine appears to inhibit both types [4].

In rabbits, excitation, hyperthermia and hypertension result from the combined administration of non-selective MAO inhibitor phenelzine and pethidine [3]. Although deprenyl may not be a selective MAO type B inhibitor in rabbits, it did not cause the toxic interaction with pethidine. We therefore tested both clorgyline and deprenyl in this respect.

Materials and methods

Rabbits of either sex weighing 2.2–3.0 kg received food and water *ad lib.* until the experiments. Behaviour of the animals was observed in plastic cages, one animal in each. Rectal temperature was measured with the thermocouple. Clorgyline hydrochloride (May & Baker, Dagenham), l-deprenyl hydrochloride (Chinoin, Budapest), pethidine hydrochloride, and phenelzine hydrogen sulphate (Leo AB, Hälsingborg) were freshly dissolved in sterile saline, the doses used referring to the base. Pretreatment drugs were injected subcutaneously whereas pethidine was given into the marginal ear vein.

Contents of 5HT and of 5-hydroxyindole acetic acid (5HIAA) were assayed according to Ashcroft and Sharman [9], and the NA content of the right haemisphere according to Shore & Olin [10]. The recovery of added NA in the latter method was low but the results obtained were about similar to those we have previously found with a more accurate Dowex column method [11]. The statistical significance of the differences between two means was determined by Student's *t*-test.

Results

Phenelzine and pethidine. Pethidine (1.25 mg/kg i.v.) exerted no behavioural effects but slightly lowered rectal temperature when compared to saline treated controls. Pretreatment with phenelzine (5 or 20 mg/kg s.c.) 10 hr previously to pethidine rendered the animals alert but they still were easy to handle. As seen in Table 1, pretreatment with phenelzine 5 mg/kg elevated hypothalamic 5HT and the 5HT/5HIAA ratio, and lowered hypothalamic 5HIAA, thus demonstrating a strong MAO inhibition. The NA levels in haemisphere after pethidine alone were 554 ± 31 ng/g whereas higher levels (831 ± 9 ng/g) were measured in two animals pretreated with phenelzine.

In phenelzine-treated rabbits pethidine caused excitation, tremor and nodding being the most prominent symptoms. Rectal temperature rose by 0.5° and by 2.8° in these two pretreated groups, respectively (Fig. 1, left). These rises were clearest at 60 min after pethidine. Two out of five rabbits died after pethidine injected to rabbits pretreated with 20 mg/kg of phenelzine. Just before the death the rises in rectal temperature were 3.4° and 4.4°, respectively.

Clorgyline and pethidine. Pretreatment with clorgyline (1.5 or 20 mg/kg s.c.) 10 hr before pethidine had no observable behavioural or temperature effects. Pethidine (1.25 mg/kg i.v.) caused no excitation or hyperthermia in these animals (Fig. 1, right). As seen in Table 1, clorgyline proved less potent MAO inhibitor towards 5HT than

Table 1. Hypothalamic 5HT and 5HIAA after different MAO inhibitors

Treatment	mg/kg	*n*	5HT and 5HIAA levels in hypothalamus ng/g, mean ± SE: 5HT	5HIAA	$\frac{5HT}{5HIAA}$ ratio
Controls		(8)	656 ± 49	554 ± 31	1.22 ± 0.11
Phenelzine	5	(5)	1449 ± 144†	255 ± 31†	6.91 ± 1.89
Clorgyline	5	(6)	1150 ± 31†	447 ± 61	2.74 ± 0.36
Clorgyline	20	(8)	1023 ± 19†	351 ± 22†	2.59 ± 0.12
Deprenyl	5	(5)	952 ± 100*	304 ± 40†	3.33 ± 0.58
Clorgyline + Deprenyl	1 + 1	(5)	1312 ± 79†	202 ± 42†	7.74 ± 1.67
Clorgyline + Deprenyl	5 + 5	(5)	1739 ± 176†	Nil†	

10 hr after the injection of MAO inhibitors the rabbits were given 1.25 mg/kg i.v. of pethidine and the animals were killed 2 hr later. Number of rabbits in parentheses.
Significant differences from controls are marked, *$P < 0.05$, †$P < 0.001$.
For more details see text.

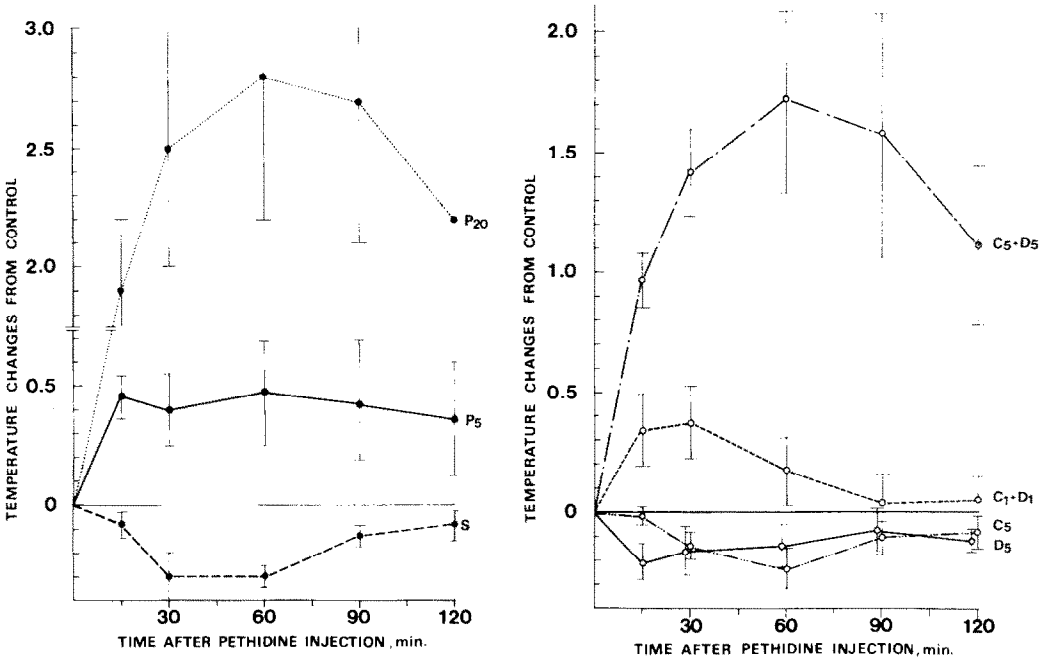

Fig. 1. Changes in rectal temperature after an i.v. injection of pethidine to rabbits pretreated with saline (S), phenelzine (P), clorgyline (C), or deprenyl (D), alone or in combinations. Mean values ± SE of 5–8 rabbits are given. For more details see text.

phenelzine was but it significantly elevated 5HT and lowered 5HIAA as compared with controls. The doses of 5 or 20 mg/kg of clorgyline did not differ essentially from each other in this respect since their 5HT/5HIAA ratios proved similar. The NA levels of brain haemisphere (750 ± 65 ng/g) measured after 5 mg/kg of clorgyline were higher than those found in controls.

Deprenyl and pethidine. Pretreatment with deprenyl (1,5 or 20 mg/kg s.c.) 10 hr before pethidine had no constant behavioural effect, but some rabbits were alert after 5 or 20 mg/kg of deprenyl. As seen in Table 1, deprenyl 5 mg/kg proved a moderate MAO inhibitor towards 5HT, elevating the 5HT/5HIAA ratio about as 5 mg/kg of clorgyline did. It showed a clearer effect in the 5HIAA reduction than in the 5HT elevation. Contrary to the findings with phenelzine and clorgyline, the NA levels in haemisphere (493 ± 50 ng/g) measured in five rabbits remained similar to those measured in control rabbits, possibly due to the complex action of deprenyl on NA neurons [12].

Pethidine showed variable effects in these animals. As a rule, in those animals who were alerted after pretreatment with deprenyl (5 or 20 mg/kg), pethidine caused slight excitation and hyperthermia. The mean change in rectal temperature is illustrated in Fig. 1. If excitation and hyperthermia occurred, they seemed to last shorter time interval than after phenelzine + pethidine. In some animals the response to pethidine was also tested 24, 48 and 72 hr after the administration of deprenyl, and the results were similar to those seen after the 10-hr time interval.

Clorgyline + deprenyl + pethidine. Combined pretreatment with clorgyline (1 or 5 mg/kg s.c.) together with deprenyl (1 or 5 mg/kg s.c.) given 10 hr previously to pethidine, rendered the animals alert without changing the rectal temperature, thus resembling the situation after pretreatment with phenelzine. As seen in Table 1, pretreatment with clorgyline + deprenyl, 1 mg/kg each, inhibited MAO about to the same extent as 5 mg/kg of phenelzine did. When using 5 mg/kg of clorgyline and deprenyl each, a complete inhibition of MAO towards 5HT was seen since 5HT levels were high and 5HIAA levels fell to zero. The effects of this combination on the hypothalamic 5HT and 5HIAA were significantly ($P < 0.05$ to 0.001) superior to those seen after clorgyline or deprenyl alone or after their combination in low doses (1 + 1 mg/kg). The haemisphere NA levels (611 ± 55 ng/g) measured after the combination of 5 + 5 mg/kg of drugs did not differ from the control values.

Pethidine caused some restlessness and elevated rectal temperature ($P < 0.01$) in animals pretreated with 1 mg/kg of both MAO inhibitors (Fig. 1). In animals pretreated with 5 mg/kg of both clorgyline and deprenyl pethidine evoked a characteristic toxic reaction always seen in animals after the injection of pethidine to phenelzine-treated rabbits: nodding, restlessness, tremor, and sometimes jumping were evident in every animal, and marked hyperthermia with maximum at 30–90 min after pethidine followed. Three out of ten animals died. At death the mean elevation of temperature was 4.7°.

Discussion

The present data confirm the phenelzine-induced elevation of hypothalamic 5HT associated with the toxic interaction between phenelzine and pethidine [3,11]. Also clorgyline, a selective inhibitor of MAO type A, moderately elevated the brain levels of 5HT and NA but the toxic effects after pethidine did not appear in these animals. Since substantial amounts of 5HIAA still were formed even after 20 mg/kg of clorgyline (Table 1), the inhibition of MAO obviously remained incomplete in spite of elevated brain 5HT and of the documented high potency of clorgyline previously found on the rabbit tissues *in vitro* [8] and on the rat brain *in vivo* [7]. This finding agrees with the recent view that recommends caution when applying *in vitro* data to *in vivo* conditions [13]. Actually, few data are available on the actions of selective MAO inhibitors in rabbits.

Pretreatment of rabbits with non-selective deprenyl elevated hypothalamic 5HT and lowered 5HIAA without pethidine toxicity. Administration of both deprenyl and clorgyline even in low doses provided high levels of 5HT and low levels of 5HIAA, and provoked toxic symptoms after pethidine. This combined effect is stronger than their

effect on the brain dopamine in rats [7], and it may not be just an additive effect on MAO type A. The finding rather supports the view that the strict subdivision of MAO into types A and B may not work as strictly separated *in vivo*. Green & Youdim [14] as well as Squires & Buus Lassen [15] have demonstrated that the characteristic tryptophan syndrome in rats is obtained after inhibition of MAO B by deprenyl and MAO A by clorgyline whereas either drug alone failed to produce this syndrome. They concluded that after inhibition of MAO A by clorgyline a further oxidation of 5HT by MAO B occurs if needed. In rabbit hypothalamus there are two forms of MAO B, but generally MAO is more active towards 5HT than PEA [16]. Our results with rabbits do not disagree with the data quoted above. A pharmacokinetic interaction of deprenyl and clorgyline is possible.

It is also possible that mediators other than 5HT are needed for the toxicity of pethidine in these circumstances. Deprenyl inhibits not only MAO but also the uptake of NA on the noradrenergic neurons [12] and these two mechanisms together are well known to provoke to toxic actions and interactions.

We have previously demonstrated that high 5HT levels *per se* are not sufficient since the phenelzine pethidine toxic interaction was absent in rabbits pretreated with *p*-chlorophenylalanine and 5HTP resulting in extremely high hypothalamic 5HT levels [11]. *p*-Chlorophenylalanine can desensitize central monoamine receptors since it is a weak agonist on peripheral 5HT receptors [17]. To answer the question whether other monoamines (PEA, dopamine etc.) contribute to the toxic interaction, needs a thorough analysis of their kinetics *in vivo* condition. Experiments with PEA and dopamine are in progress.

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