J. Pharm. Pharmac., 1978, 30, 36–40 Received May 23, 1977

Modification by monoamine oxidase inhibitors of the analgesic, hypothermic and toxic actions of morphine and pethidine in mice

REGINA BOTTING*, SUSAN BOWER, C. T. EASON, P. H. HUTSON AND LINDA WELLS

Biology Department, North East Surrey College of Technology, Ewell, Surrey, U.K.

A single injection of phenelzine 100 mg kg^{-1} given 18 h before, decreased the analgesia and hypothermia induced by morphine, but potentiated the analgesic and hypothermic effects of pethidine, when the analgesics were administered either intraperitoneally or intracerebroventricularly. The modification of pethidine analgesia and hypothermia, but not morphine analgesia, was antagonized by methysergide (10 mg kg^{-1}, s.c.). The LD50 of pethidine, but not that of morphine, was 30–40% lower in mice treated with phenelzine tranylcypromine or iproniazid 6 h before the test. The increased lethality of a single dose of pethidine induced by phenelzine was also prevented by methysergide. Pretreatment of mice with 100 mg kg^{-1} phenelzine was followed by a significant rise in both brain tryptophan and 5-hydroxytryptamine (5-HT) concentrations which lasted for 24 h. Therefore, the changes in pethidine effects could have been due to raised brain tryptophan and 5-HT concentrations.

The occurrence of a toxic interaction during the concurrent administration of pethidine and monoamine oxidase inhibitor drugs (MAOIs) is well established (Taylor, 1962). Such a dramatic interaction does not seem to occur between MAOIs and morphine however. It would thus appear that the actions of pethidine and morphine are affected differently by MAOIs and this supposition has been investigated experimentally.

Jounela & Mattila (1968) reported that in mice pethidine analgesia was increased and morphine analgesia decreased by prior injection of the MAOI phenelzine, and similarly the LD50 of pethidine, but not that of morphine, was reduced by this MAOI (Jounela, 1970). Further, Penn & Rogers (1971) showed that pethidine, but not morphine, produced hyperpyrexia in rabbits pretreated with pargyline. Conversely, Rogers & Thornton (1969) described an increase in toxicity to both pethidine and morphine in mice pretreated with either tranylcypromine or iproniazid. Mustala & Jounela in 1966 had reported a similar finding in mice pretreated with pargyline.

The following investigation was initiated to establish whether the actions of both morphine and pethidine in mice would be similarly affected by previous administration of MAOIs and whether the interaction could be attributed to interference with the metabolism of the analgesics as suggested by Yeh & Mitchell (1971) or rather to alteration in metabolism of brain 5-hydroxytryptamine.

* Correspondence.

METHODS

Male albino mice (CPLF strain, Anglia Laboratories) 16–28 g were given monoamine oxidase inhibitors subcutaneously and narcotic analgesic drugs intraperitoneally or intracerebroventricularly. In all cases an equivalent volume of saline was injected into the control group.

Analgesia

Analgesia was assessed by placing the mice on a hot plate maintained at 56° and measuring the time taken for animals to lick their paws (Janssen & Jageneau, 1957). Groups of eight mice per dose level of analgesic were used and the reaction time measured to the nearest 0·5 s. Phenelzine (100 mg kg^{-1}) was administered 18 h before the analgesic test. Reaction time was measured 10 min before and at time of maximum analgesia (20 min after i.p. administration of 6, 12 or 24 mg kg^{-1} pethidine or morphine and 5 min after i.c.v. injections of 0·25, 0·5 or 1 mg kg^{-1} pethidine or 0·025, 0·05 or 0·1 mg kg^{-1} morphine). The reaction times measured in this way showed wide day to day variations so comparisons were made only on concurrently tested groups of mice. Drugs were administered in 0·1 ml saline per 10 g body weight intraperitoneally and in a volume of 10 μl of saline intracerebroventricularly (method of Haley & McCormick, 1957). The distribution of solutions after intracerebroventricular injection was checked by section of the brain after administration of indian ink.

Rectal temperature

Rectal temperatures were recorded on a galvano-

meter by means of a probe inserted to approximately 1 cm. The temperatures of mice treated with phenelzine (100 mg kg^{-1}, s.c.) 18 h before and either pethidine (24 mg kg^{-1} and 100 mg kg^{-1}, i.p.) or morphine (6 mg kg^{-1}, i.p.), were compared with the temperatures of mice given the analgesics alone. Temperatures were recorded before and up to 90 min after the administration of the analgesic. Ambient temperature was 22°.

Acute toxicity

The LD50 concentrations (95% confidence limits) were determined by the method of Lichfield & Wilcoxon (1949) on groups of 8 mice observed for 2 h at 22°. Phenelzine (100 mg^{-1} kg), tranylcypromine (15 mg kg^{-1}) and iproniazid (500 mg kg^{-1}) were administered subcutaneously 6 h before the intraperitoneal administration of the analgesic.

The effect of methysergide on pethidine lethality after phenelzine was measured on a single dose of the analgesic (115 mg kg^{-1}).

Measurement of brain 5-HT and tryptophan

Mice were killed by cervical dislocation. The brains were removed and immediately frozen on solid carbon dioxide and stored at −20°. Each brain was assayed for 5-HT by the method of Curzon & Green (1970) and for tryptophan in the aqueous phase produced by the HCl–iso-octane extraction essentially using the fluorimetric method of Denckla & Dewey (1967). Determinations of 5-HT and tryptophan were made up to 24 h after subcutaneous administration of phenelzine (100 mg kg^{-1}). *Drugs used.* phenelzine base (Warner), morphine sulphate (Macfarlane Smith Limited), pethidine hydrochloride (Roche), tranylcypromine sulphate (SKF), iproniazid phosphate (Sigma), methysergide maleate (Sandoz) 5-hydroxytryptamine creatine sulphate (May and Baker Limited) and L-tryptophan (Sigma).

RESULTS

Analgesia

When pethidine and morphine were administered intraperitoneally or intracerebroventricularly to mice which had been given 100 mg kg^{-1} phenelzine 18 h previously, the analgesic effect of pethidine was increased and that of morphine reduced (Figs 1 and 2).

The administration of methysergide 10 mg kg^{-1} 30 min before intracerebroventricular injection of the analgesics, prevented the potentiation of the pethidine effect but did not affect the reduction of morphine analgesia (Table 1). Neither phenelzine nor methysergide alone altered reaction time compared with control values.

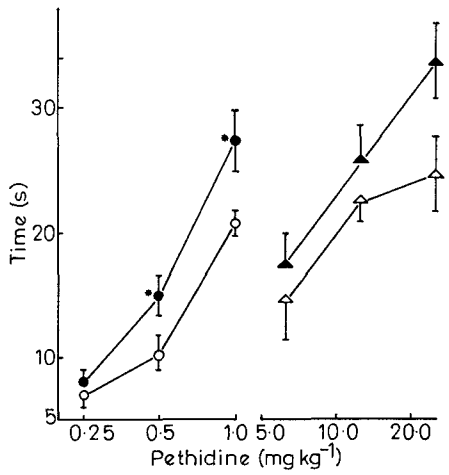

FIG. 1. Potentiation of pethidine (mg kg^{-1}) analgesia by phenelzine 100 mg kg^{-1}, subcutaneously given 18 h before. Pethidine (i.p.) (△); pethidine (i.p.) after phenelzine (▲); pethidine (i.c.v.) (○); pethidine (i.c.v.) after phenelzine (●). Ordinate: Analgesia equivalent to reaction time (s). Each point is a mean of 8 determinations, vertical bars show the standard error. * Significant at $P < 0.05$.

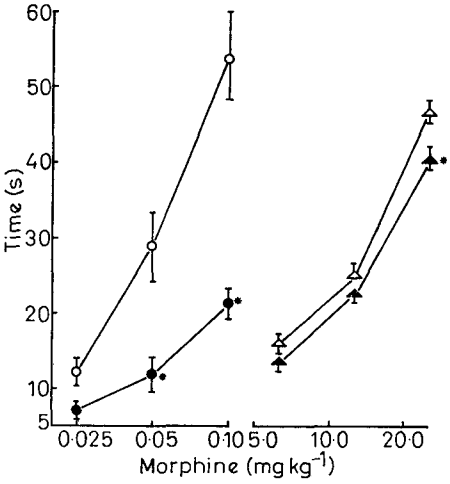

FIG. 2. Inhibition of morphine (mg kg^{-1}) analgesia by phenelzine 100 mg kg^{-1}, subcutaneously given 18 h before. Morphine (i.p.) (△); morphine (i.p.) after phenelzine (▲); morphine (i.c.v.) (○); morphine (i.c.v.) after phenelzine (●). Ordinate: Analgesia equivalent to reaction time (s). Each point is a mean of 8 determinations, vertical bars show the standard error. * Significant at $P < 0.05$.

Rectal temperature

Treatment with phenelzine (100 mg kg^{-1}) potentiated the hypothermia caused by pethidine and reversed

Table 1. *The effect of methysergide* (10 *mg kg*$^{-1}$) *on change in analgesia to morphine and pethidine produced by pretreatment of mice with phenelzine* (100 *mg kg*$^{-1}$).

Treatment	Reaction time (s ± s.e.m.) Pethidine 1 mg kg^{-1}, i.c.v.	Morphine 0·1 mg kg^{-1}, i.c.v.
Analgesic only	19·4 ± 1·9	30·1 ± 3·6
Methysergide + analgesic	17·8 ± 2·3	29·1 ± 2·2
Phenelzine + analgesic	26·8 ± 1·7*	19·3 ± 3·0*
Phenelzine + methysergide + analgesic	15·4 ± 3·6	20·4 ± 4·3

* Significant $P < 0·05$ 8 mice per group.

that produced by morphine (Fig. 3). The administration of methysergide (10 mg kg^{-1}) 60 min before injection of pethidine (i.p.), prevented the potentiation of the pethidine effect (Fig. 4).

Acute toxicity

The LD50 of pethidine was reduced by 30–40% in mice predosed with phenelzine (100 mg kg^{-1}), tranylcypromine (15 mg kg^{-1}) or iproniazid 500 mg kg^{-1}) 6 h before. However, the LD50 of morphine was not altered by pretreatment with these monoamine oxidase inhibitors (Table 2). Administration of methysergide (10 mg kg^{-1}, s.c.) 60 min before pethidine (115 mg kg^{-1}) after phenelzine treatment, protected the mice (deaths 0/5) from the effects of this previously lethal drug combination (deaths 5/5). There were no deaths after pethidine alone.

Brain 5-HT and tryptophan concentrations after injection of phenelzine

Brain 5-HT concentrations increased after injection of phenelzine, reaching a maximum in 6 h. Tryptophan concentrations also rose, the maximum occurring 2 h after phenelzine. The brain concentrations of both tryptophan and 5-HT were still significantly raised above normal 18 h after phenelzine (Fig. 5).

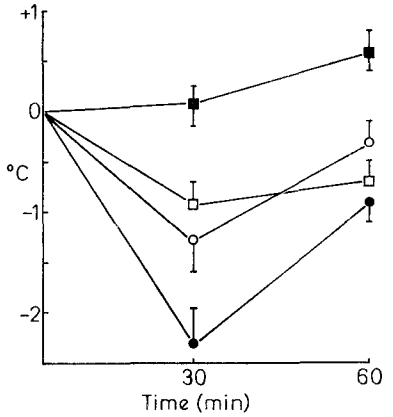

FIG. 3. Potentiation and inhibition of pethidine and morphine hypothermia by phenelzine 100 mg kg^{-1}, subcutaneously administered 18 h before. Pethidine 24 mg kg^{-1} (i.p.) (○); phenelzine and pethidine 24 mg kg^{-1} (i.p.) (●); morphine 6 mg kg^{-1} (i.p.) (□); phenelzine and morphine 6 mg kg^{-1} (i.p.) (■). Each point is a mean of 8 determinations. Change in rectal temperature (°C) was obtained by taking the difference between test values and the mean of 8 control mice injected with saline. Vertical bars show the standard error. Abscissa: Time after administration of analgesic. Phenelzine on its own did not alter body temperature. Phenelzine treatment produced a significant difference in the hypothermia induced by the analgesics ($P < 0·05$). The hypothermia caused by morphine and phenelzine at 60 min was significantly different from saline-treated animals ($P < 0·05$).

DISCUSSION

The confusion in published reports on the interactions between narcotic analgesics and MAOIs can be attributed in part to the variable effects of different dosing schedules and also to different effects of pethidine and morphine. Thus in our mice,

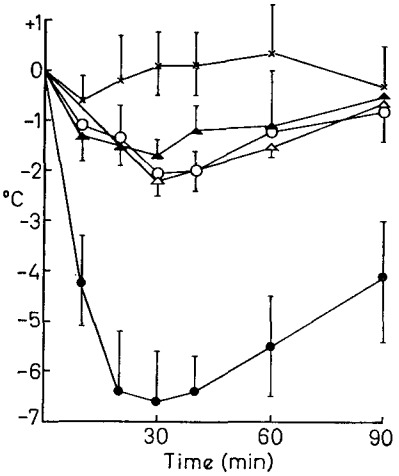

FIG. 4. Reversal of the potentiation of pethidine hypothermia with phenelzine 100 mg kg^{-1}, subcutaneously by methysergide 10 mg kg, (i.v.) administered 60 min before. Saline 10·0 ml kg^{-1} (i.p.) (×); pethidine 100 mg kg^{-1} (i.p.) (○); phenelzine and pethidine 100 mg kg^{-1} (i.p.) (●); phenelzine and methysergide (△); phenelzine, methysergide and pethidine 100 mg kg^{-1} (i.p.) (▲). Each point is a mean of 5 determinations, vertical bars show the standard error. Abscissa: Time after adminstration of analgesic. Ordinate: Change in rectal temperature (°C).

Table 2. *LD50 (95% confidence limits) of analgesic drugs in mice pretreated with monoamine oxidase inhibitors.*

Pretreatment	Pethidine LD50 mg kg^{-1}	Morphine LD50 mg kg^{-1}
Saline	135 (127–144)	370 (340–441)
Phenelzine 100 mg kg^{-1}	80 (69–94)	400 (285–560)
Tranylcypromine 15 mg kg^{-1}	80 (66–98)	375 (300–468)
Iproniazid 500 mg kg^{-1}	90 (80–102)	350 (269–455)

Doses of analgesics expressed as mg base.

a single administration of phenelzine potentiated the analgesia and hypothermia produced by pethidine, whereas the effects of morphine were uniformly antagonized by treatment with phenelzine.

The discrimination by phenelzine between the actions of pethidine and morphine might be due to increased metabolism of morphine subsequent to induction of hepatic enzymes. Yeh & Mitchell (1971) suggested that induction of glucuronyl transferase occurred on treatment with some MAOIs, and that this explained the reversal of morphine analgesia in animals so treated. This is unlikely to be the explanation in the present experiments since intracerebroventricular injections of morphine and pethidine produced analgesic effects within 5 min which were also reversed (morphine) or potentiated (pethidine) by acute treatment with phenelzine. It is thus unlikely that an alteration in systemic metabolism of the analgesics after administration of phenelzine could account for the difference in effect of phenelzine on the action of morphine or pethidine.

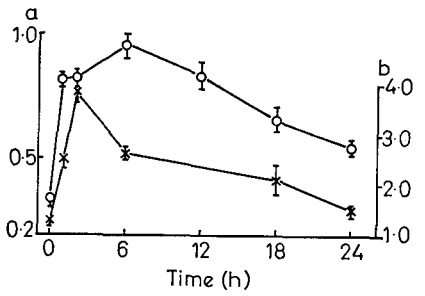

FIG. 5. Effect of phenelzine 100 mg kg^{-1}, subcutaneously on brain a—5-HT (○; μg g^{-1}) and b—tryptophan (×; μg g^{-1}) concentrations in mice. Each point is a mean of 6 determinations, vertical bars show the standard error. All determinations were greater than control levels ($P < 0.05$).

Methysergide (10 mg kg^{-1}) administered before the narcotic analgesics, reversed the increase in pethidine analgesia and hypothermia but not the decrease in morphine analgesia. Assuming a selective action of methysergide on 5-HT receptors in the central nervous system, this indicates that a 5-HT mechanism might be responsible at least for the enhancement of the pethidine effect by phenelzine.

The concentration of brain 5-HT and its precursor tryptophan was significantly raised above control values 18 h after a single dose of phenelzine. The potentiation of pethidine analgesia and hypothermia may indicate that pethidine is more dependent on the availability of 5-HT for its action than morphine. This is confirmed by the antagonism by methysergide of the potentiation of pethidine by phenelzine.

Rogers & Thornton (1969) found that the maximum increase in acute toxicity to pethidine occurred 4–6 h after a single dose of MAOI. They also showed that this correlated with the maximum increase in brain 5-HT concentrations. This was confirmed in our experiments where maximum 5-HT concentrations occurred (and pethidine toxicity was consistently altered) 6 h after administration of MAOIs. Nevertheless, unlike the previous workers we could not show a similar increase in morphine toxicity with any of the MAOIs studied. This is in accord with the results of Jounela (1970) who reported an enhanced toxicity of pethidine but not of morphine in mice 10 h after phenelzine treatment. As with pethidine analgesia and hypothermia, methysergide abolished the increased lethality of pethidine induced by phenelzine.

The maximum rise in tryptophan concentrations preceded the peak of 5-HT increase by at least 2 h. This may indicate that phenelzine raised the amine concentrations by another mechanism in addition to the inhibition of MAO. This other mechanism may involve raising brain tryptophan concentrations by displacement of tryptophan from its binding to plasma albumin (Hutson, unpublished). Rate of synthesis of brain 5-HT is probably dependent on the availability of free tryptophan from plasma (Curson, Joseph & Knott, 1972).

These results indicate that pethidine and morphine may exert their analgesic and toxic effects by different mechanisms, and could explain the absence of clinical reports of a toxic interaction between morphine and MAOIs. Other factors which may contribute to the lack of toxicity of the morphine and MAOI combination is the wide margin between analgesic and toxic dose and the high analgesic potency of morphine. The margin between analgesic

and toxic dose of pethidine is much narrower and a single analgesic dose of pethidine in man is likely to be 10× greater than that of morphine (Goodman & Gilman 1975).

Acknowledgements

Grateful thanks are due to Mr I. A. Ramshaw for technical assistance and Mr G. M. Whitford of William R. Warner & Co. Limited for the generous gift of phenelzine.

REFERENCES

CURZON, G. & GREEN, A. R. (1970). *Br. J. Pharmac.*, **39**, 653–655.

CURZON, G., JOSEPH, M. H. & KNOTT, P. J. (1972). *J. Neurochem.*, **19**, 1967–1974.

DENCKLA, W. D. & DEWEY, H. K. (1967). *J. Lab. clin. Med.*, **69**, 160–169.

GOODMAN, L. S. & GILMAN, A. (1975). *The Pharmacological Basis of Therapeutics*, pp. 258, 265. N.Y.: Macmillan Publishing Co. Inc.

HALEY, T. J. & MCCORMICK, W. G. (1957). *Br. J. Pharmac. Chemother.*, **12**, 12–15.

JANSSEN, P. J. A. & JAGENEAU, A. H. (1957). *J. Pharm. Pharmac.*, **9**, 381–400.

JOUNELA, A. J. (1970). *Ann. Med. exp. Fenn.*, **48**, 261–265.

JOUNELA, A. J. & MATTILA, M. J. (1968). *Ibid.*, **46**, 66–71.

LICHFIELD, J. T. & WILCOXON, F. (1949). *J. Pharmac. exp. Ther.*, **96**, 99–113.

MUSTALA, O. O. & JOUNELA, A. J. (1966). *Ann. Med. exp. Fenn.*, **44**, 395–396.

PENN, R. G. & ROGERS, K. J. (1971). *Br. J. Pharmac.*, **42**, 485–492.

ROGERS, K. J. & THORNTON, J. A. (1969). *Ibid.*, **36**, 470–480.

TAYLOR, D. C. (1962). *Lancet*, **2**, 401.

YEH, S. Y. & MITCHELL, C. L. (1971). *J. Pharmac. exp. Ther.*, **179**, 642–651