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Role of brain monoamines in the fatal hyperthermia induced by pethidine or imipramine in rabbits pretreated with a monoamine oxidase inhibitor

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Summary

1. The intravenous infusion of pethidine or imipramine, in doses of 5 mg/kg, caused fatal hyperpyrexia in rabbits premedicated with pargyline.

2. The drug interaction was not antagonized when either reserpine or α-methyl-*p*-tyrosine were administered with pargyline. Neither reserpine nor α-methyl-*p*-tyrosine prevented the rise in brain stem 5-hydroxytryptamine content following monoamine oxidase inhibition, although the increase in catecholamines normally produced by pargyline was prevented.

3. The development of fatal hyperthermia was completely prevented when rabbits were treated with *p*-chlorophenylalanine prior to pargyline premedication. In these animals, the concentration of brain stem catecholamines, but not 5-hydroxytryptamine, was increased.

4. The results indicate that the hyperthermia evoked by pethidine or imipramine in combination with monoamine oxidase inhibitors can take place only in the presence of raised concentrations of 5-hydroxytryptamine in the brain stem.

Introduction

In patients receiving treatment with monoamine oxidase (MAO) inhibitors, therapeutic doses of pethidine or tricyclic antidepressants have caused severe and often fatal toxic reactions, characterized by symptoms which include excitement and a pronounced hyperthermia (Goldberg, 1964 ; Sjöqvist, 1965). Similar hyperthermic reactions occur in rabbits given these drug combinations (Nymark & Nielsen, 1963 ; Loveless & Maxwell, 1965). The interactions have been attributed to a decreased metabolism of the analgesic or antidepressant drug, since MAO inhibitors are known to exert non-specific inhibiting effects on detoxifying enzyme systems in the liver. This seems unlikely to be the sole mechanism responsible for the drug interaction since high doses of pethidine or imipramine do not result in the same pharmacological sequelae as those produced by the drug combination (Loveless & Maxwell, 1965 ; Penn & Rogers, 1971). In view of the unusual features of the reaction it seems more likely that it may result from the raised concentrations of brain catechol- or indole-amines resulting from MAO inhibition.

This paper describes some attempts to modify the toxic reaction in rabbits by pretreating them with drugs that selectively lower the concentration of brain monoamines. A preliminary report of this work has been presented to the British Pharmacological Society (Gong & Rogers, 1971).

Methods

The experiments were carried out on male Californian rabbits weighing between 1·5 and 2·5 kg. Preliminary studies showed that the injection of two doses of the MAO inhibitor pargyline, 25 mg/kg subcutaneously, with an interval of one day between, was a suitable pretreatment in that the subsequent injection of pethidine or imipramine, 5 mg/kg, invariably evoked a hyperthermic response. The pethidine or imipramine was injected 18 h after the last dose of pargyline.

Prior to the injection of pethidine or imipramine, the MAO inhibitor was given to otherwise untreated animals or to rabbits treated with monoamine depleting drugs according to the following dosage schedules: Four doses of α-methyl-*p*-tyrosine, 80 mg/kg i.p., were given at 12 hourly intervals on the 2 days preceding the injection of pethidine or imipramine. Reserpine, 0·5 mg/kg i.p. daily, was injected for 2 consecutive days before the recording day. *p*-Chlorophenylalanine, 125 mg/kg, was injected (i.p.) daily on the three days prior to pargyline pretreatment.

On the day of the experiment, the rabbits were placed in head stocks to permit the recording of rectal temperature. A period of 30 minutes was allowed for the rabbit to settle in the stocks before pethidine or imipramine was infused slowly into the marginal ear-vein at a rate of 1 (mg/kg)/minute. Rectal temperature was measured by a thermistor probe inserted 8–10 cm into the rectum. The temperature was recorded on an electric thermometer (Light Laboratories, Brighton). Four animals were used in each group. Separate groups of rabbits were killed for the biochemical studies.

Measurement of brain stem monoamines

Eight control rabbits and 12 drug-treated rabbits, 4 groups of 3, were used. The animals were killed by air embolism, and the brains minus cerebellum and cortex were rapidly removed and frozen in liquid nitrogen until used for assay. The monoamines were extracted simultaneously using the method of Shore & Olin (1958). Portions of the final acid extract were used for the estimation by fluorimetry of noradrenaline (Anton & Sayre, 1962), dopamine (Carlsson & Waldeck, 1958) and 5-hydroxytryptamine (Bogdanski, Pletscher, Brodie & Udenfriend, 1956).

The following drugs were used; imipramine hydrochloride (Geigy Ltd.), pargyline hydrochloride (Abbott Laboratories), pethidine hydrochloride, reserpine, *p*-chlorophenylalanine and α-methyl-*p*-tyrosine methyl ester. Reserpine was dissolved in 5% acetic acid; *p*-chlorophenylalanine was suspended in 0·5% Tween 80; the remaining compounds were dissolved in Sodium Chloride Injection B.P. Drug doses are given in terms of the salts where these were used.

Results

Body temperature effects

Neither pethidine nor imipramine, each 5 mg/kg, produced any significant changes in the body temperature of normal rabbits. The effects of pethidine and imipramine on the rectal temperature of rabbits pretreated with pargyline in combination with monoamine depleting agents, are illustrated in Figure 1.

In rabbits pretreated with pargyline alone (Fig. 1a), injection of either pethidine or imipramine rapidly evoked hyperthermia. This was accompanied by bouts of

shivering, motor restlessness and profuse salivation. The animals died in hyperthermia some 50–60 min after injection.

An attempt was made to antagonize the interaction by pretreating rabbits with reserpine in combination with the MAO inhibitor. Reserpine did not antagonize the drug interaction since all the animals developed the hyperthermic response following the injection of pethidine or imipramine (Fig. 1b).

Similarly, the drug interaction was not antagonized by the administration of α-methyl-*p*-tyrosine in combination with pargyline (Fig. 1c). All the animals in these groups developed hyperthermia following pethidine or imipramine injection, although in 2 rabbits (one from each group) the hyperthermia was not fatal.

The toxic reaction was completely prevented by *p*-chlorophenylalanine, none of the rabbits exhibiting excitement or hyperthermia after pethidine or imipramine (Fig. 1d).

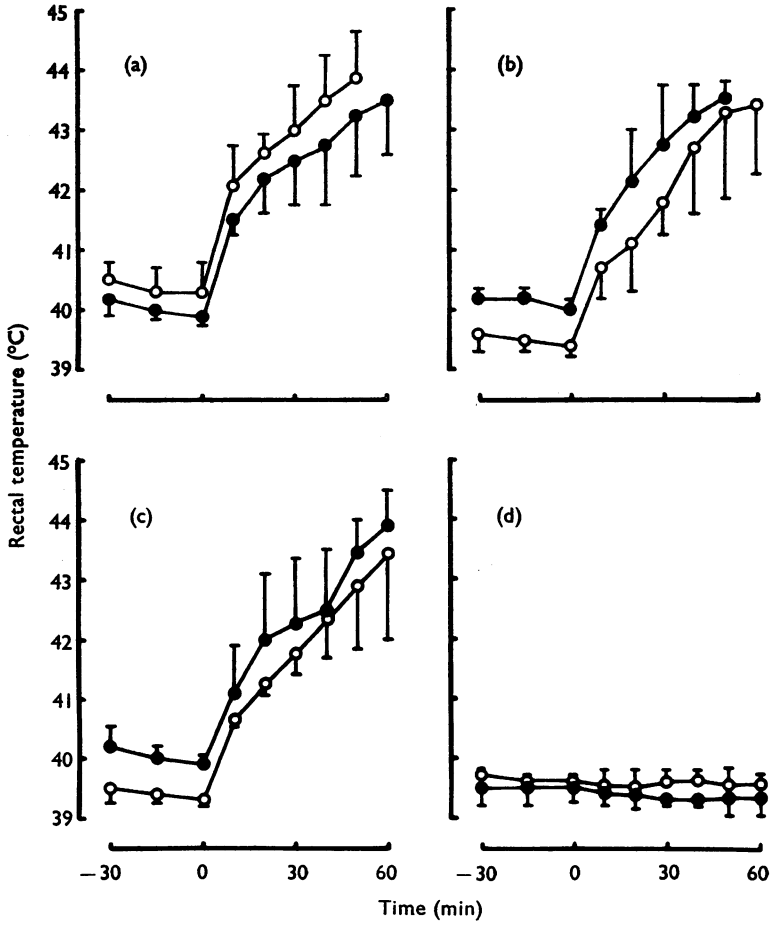

FIG. 1. Effect of pethidine (○——○) and imipramine (●——●), given by intravenous infusion, on the rectal temperature of rabbits pretreated with: (a) pargyline, (b) pargyline and reserpine, (c) pargyline and α-methyl-*p*-tyrosine and (d) pargyline and *p*-chlorophenylalanine. Drug dosage schedules are described in **Methods.** Each curve represents the mean response from four rabbits. The vertical lines indicate the S.E.M.

Effects on brain stem monoamines

In the brain stem of control rabbits, the mean concentrations (±S.E.M.) of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) were 0·46±0·01 μg/g, 0·34±0·01 μg/g and 0·59±0·03 μg/g respectively. As expected, pargyline increased the concentration of all three monoamines studied (Fig. 2a).

Reserpine alone caused a fall in the amine concentrations (Fig. 2b). In rabbits given reserpine and pargyline, the content of brain stem noradrenaline was similar to that of the controls, but the dopamine was depleted, whereas the concentration of 5-HT was increased to a value similar to that found in rabbits treated with pargyline alone. Other workers have shown that, in the presence of reserpine, brain 5-HT content is more readily modified by MAO inhibitors than that of the catecholamines (Green & Erickson, 1962).

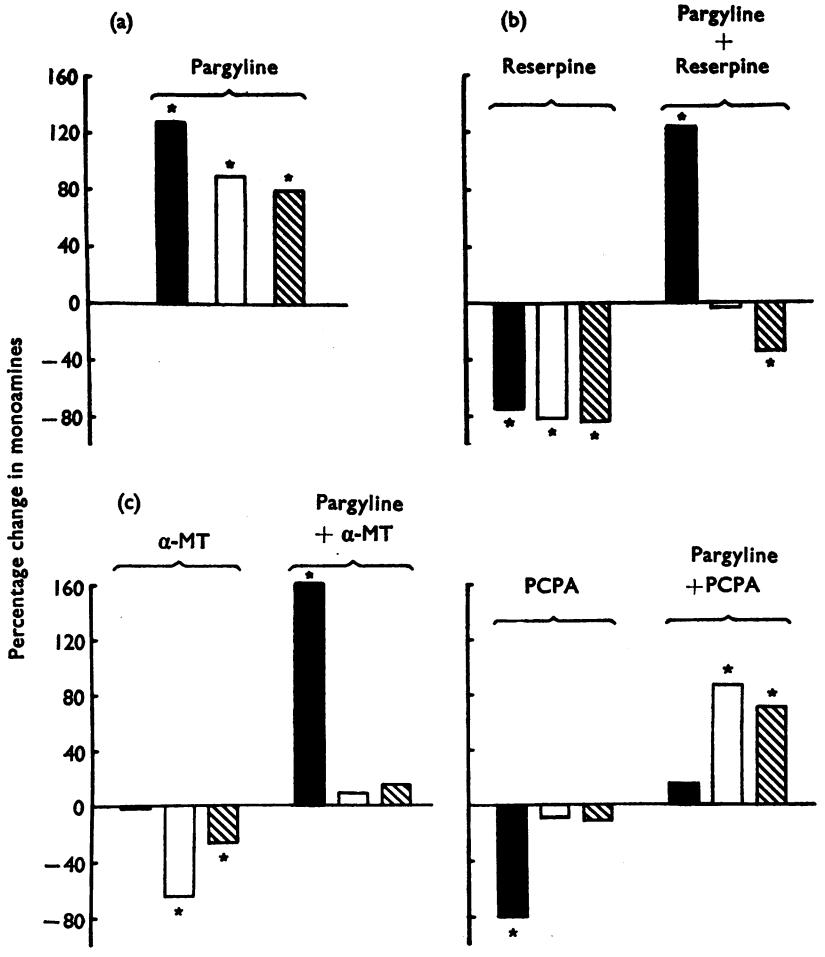

FIG. 2. Percentage changes in the concentration of 5-hydroxytryptamine (■), noradrenaline (□) and dopamine (▩) in the brain stem of rabbits treated with: (a) pargyline alone, (b) pargyline and reserpine, (c) pargyline and α-methyl-*p*-tyrosine (α-MT), and (d) pargyline and *p*-chlorophenylalanine (PCPA). Drug dosage schedules are described in **Methods.** Control values are given in **Results.** Each value is the average from three rabbits. Asterisks indicate values significantly different from controls ($P<0·05$).

The tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine, selectively decreased the concentration of brain stem catecholamines without affecting the concentration of 5-HT (Fig. 2c). As with reserpine, when pargyline was given in conjunction with α-methyl-*p*-tyrosine, brain stem 5-HT content was again increased whereas the catecholamine concentrations merely returned to control values.

p-Chlorophenylalanine, a tryptophan hydroxylase inhibitor, selectively depleted brain stem 5-HT without significantly reducing the catecholamine concentration (Fig. 2c). However, when pargyline was given after *p*-chlorophenylalanine treatment the catecholamine concentrations increased to values similar to those produced by pargyline alone, but the large increase in 5-HT normally produced by pargyline was completely abolished.

Discussion

There is considerable evidence that the hypothalamic control of body temperature depends upon the relative concentrations or rates of release of 5-HT and noradrenaline (Feldberg & Myers, 1964 ; 1965). It has been suggested that changes in the concentration of brain monoamines may be responsible for the hyperthermia produced by imipramine or pethidine in rabbits previously treated with a MAO inhibitor (Nymark & Nielsen, 1963 ; Loveless & Maxwell, 1965). Both noradrenaline (Cooper, Cranston & Honour, 1965) and suitable doses of 5-hydroxytryptamine (Canal & Ornesi, 1961 ; Jacob, Girault & Peindaries, 1972), when injected intracerebrally increase body temperature in rabbits. Thus the primary purpose of the present study was to determine if one or more of the brain monoamines might play a significant role in the hyperthermic reaction produced in rabbits by pethidine or imipramine in combination with a MAO inhibitor.

An initial attempt was made to antagonize the drug interaction by pretreating the rabbits with reserpine. However, reserpine simultaneously depletes catecholamine and indoleamine stores in the central nervous system, and thus does not permit distinction between the separate role of each class of amine in the drug interaction. To make this distinction, α-methyl-*p*-tyrosine and *p*-chlorophenylalanine which selectively deplete catecholamines (Spector, Sjoerdsma & Udenfriend, 1965) and 5-HT (Koe & Weissman, 1966) respectively, were administered.

Both reserpine and α-methyl-*p*-tyrosine failed to antagonize the hyperthermic reaction, and these agents also failed to prevent the rise in brain stem 5-hydroxytryptamine content following MAO inhibition, although the increase in catecholamines normally produced by pargyline was prevented. On the other hand, after the administration of pargyline to *p*-chlorophenylalanine-treated rabbits, catecholamines, but not 5-hydroxytryptamine, accumulated in the brain stem. In these animals the hyperthermic reaction to pethidine or imipramine was completely prevented. These findings indicate, therefore, that the interaction between pethidine or imipramine and MAO inhibitors can take place only in the presence of raised concentrations of cerebral 5-hydroxytryptamine.

Antagonism by the α-adrenoceptor blocking drug dibenzyline. (Dixit, Dhasmana, Sinha & Bhargava, 1970) suggested involvement of catecholamines in the fatal hyperpyrexia due to a MAO inhibitor-imipramine combination. However, another α-adrenoceptor blocking drug, phentolamine, does not prevent the hyperthermic response (Jounela, 1970a ; K.J.R. unpublished observations), and more recently,

Sinclair (1972) using receptor blocking agents and amine precursors has provided other evidence favouring the involvement of 5-hydroxytryptamine in the development of the hyperpyrexic response.

Studies in this laboratory (Rogers & Thornton, 1969; Rogers, 1971) and elsewhere (Gessner & Soble, 1970; Jounela, 1970b) have shown that the increased toxicity of pethidine in mice pretreated with a MAO inhibitor may be related specifically to an increased concentration of brain 5-hydroxytryptamine, thus also providing evidence of a relation between brain 5-hydroxytryptamine content and this drug interaction.

The precise mechanism involved in the interaction remains to be elucidated. It is known that tricyclic antidepressants are potent inhibitors of the neuronal re-uptake of monoamines. Therefore, the drug interaction might be explained if it is assumed that inhibition of monoamine re-uptake in animals pretreated with a MAO inhibitor results in larger quantities of extraneuronal amines. Using both histochemical and biochemical techniques Carlsson, Jonason, Lindqvist & Fuxe (1969) compared the ability of several tricyclic antidepressants to cause hyperthermia and excitement in rabbits given nialamide. The activity correlated well with the ability of the antidepressants to block the membrane pump of 5-hydroxytryptamine-containing neurones. Furthermore, Carlsson & Lindqvist (1969) have shown that pethidine blocks the neuronal re-uptake mechanism for cerebral 5-HT but has little effect on the re-uptake mechanism for noradrenaline. In this context, it is of interest that morphine and pentazocine show little ability to block neuronal re-uptake of 5-hydroxytryptamine (Carlsson & Lindqvist, 1969), and these two potent analgesics do not cause a toxic interaction in combination with MAO inhibitors (Penn & Rogers, 1971; Sinclair, 1972).

REFERENCES

ANTON, A. H. & SAYRE, D. F. (1962). A study of the factors affecting the aluminium oxide trihydroxy-indole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138**, 360–375.

BOGDANSKI, D. G., PLETSCHER, A., BRODIE, B. B. & UDENFRIEND, S. (1956). Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.*, **117**, 82–88.

CANAL, N. & ORNESI, A. (1961). Serotonina encefalica and ipertermia de vaccino. *Atti. Acad. med. lomb.*, **16**, 69–73.

CARLSSON, A., JONASON, J., LINDQVIST, M. & FUXE, K. (1969). Demonstration of extraneuronal 5-hydroxytryptamine accumulation in brain following membrane-pump blockade by chlorimipramine. *Brain Res.*, **12**, 456–460.

CARLSSON, A. & LINDQVIST, M. (1969). Central and peripheral monoaminergic membrane-pump blockade by some addictive analgesics and antihistamines. *J. Pharm. Pharmac.*, **21**, 460–464.

CARLSSON, A. & WALDECK, B. (1958). A fluorimetric method for the determination of dopamine (3-hydroxy-tyramine). *Acta physiol. scand.*, **44**, 293–298.

COOPER, K. E., CRANSTON, W. I. & HONOUR, A. J. (1965). Effects of intraventricular and intrahypothalamic injection of noradrenaline and 5-HT on body temperature in conscious rabbits. *J. Physiol., Lond.*, **181**, 852–864.

DIXIT, K. S., DHASMANA, K. M., SINHA, J. N. & BHARGAVA, K. P. (1970). Role of catecholamines in fatal hyperpyrexia induced by imipramine in MAOI treated rabbits. *Arch. int. Pharmacodyn.*, **188**, 86–91.

FELDBERG, W. & MYERS, R. D. (1964). Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. *J. Physiol., Lond.*, **173**, 226–237.

FELDBERG, W. & MYERS, R. D. (1965). Changes in temperature produced by micro-injections of amines into the anterior hypothalamus of cats. *J. Physiol., Lond.*, **177**, 239–245.

GESSNER, P. K. & SOBLE, A. G. (1970). Studies on the role of brain 5-hydroxytryptamine in the interaction between tranylcypromine and meperidine. *Fedn Proc.*, **29**, 685.

GOLDBERG, L. I. (1964). Monoamine oxidase inhibitors. *J. am. Med. Ass.*, **190**, 465–462.

GONG, S. N. C. & ROGERS, K. J. (1971). Role of brain monoamines in the fatal hyperthermia induced by pethidine or imipramine in rabbits pretreated with pargyline. *Br. J. Pharmac.*, **42**, 646P.

GREEN, J. & ERICKSON, R. W. (1962). Further studies with tranylcypromine (monoamine oxidase inhibitor) and its interaction with reserpine in rat brain. *Arch. int. Pharmacodyn.*, **135**, 407–425.

JACOB, J., GIRAULT, J. M. & PEINDARIES, R. (1972). Actions of 5-hydroxytryptamine and 5-hydroxytryptophan injected by various routes on the rectal temperature of the rabbit. *Neuropharmacology*, **11**, 1–16.

JOUNELA, A. J. (1970a). Influence of monoamine oxidase inhibitors on the cardiovascular action of some analgesics. *Ann. Med. exp. Fenn.*, **48**, 249–260.

JOUNELA, A. J. (1970b). Influence of phenelzine on the toxicity of some analgesics in mice. *Ann. Med. exp. Fenn.*, **48**, 261–265.

KOE, B. K. & WEISSMAN, A. (1966). *p*-Chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.*, **154**, 499–516.

LOVELESS, A. H. & MAXWELL, D. R. (1965). A comparison of the effects of imipramine, trimipramine and some other drugs in rabbits treated with a monoamine oxidase inhibitor. *Br. J. Pharmac. Chemother.*, **25**, 158–170.

NYMARK, M. & NIELSEN, J. (1963). Reactions due to the combination of monoamine oxidase inhibitors with thymoleptics, pethidine or methylamphetamine. *Lancet*, **2**, 524–525.

PENN, R. G. & ROGERS, K. J. (1971). Comparison of the effects of morphine, pethidine and pentazocine in rabbits pretreated with a monoamine oxidase inhibitor. *Br. J. Pharmac.*, **42**, 485–492.

ROGERS, K. J. (1971). Role of brain monoamines in the interaction between pethidine and tranylcypromine. *Eur. J. Pharmac.*, **14**, 86–88.

ROGERS, K. J. & THORNTON, J. A. (1969). The interaction between monoamine oxidase inhibitors and narcotic analgesics in mice. *Br. J. Pharmac.*, **36**, 470–480.

SHORE, P. A. & OLIN, J. S. (1958). Identification and chemical assay of norepinephrine in brain and other tissues. *J. Pharmac. exp. Ther.*, **122**, 295–300.

SINCLAIR, J. G. (1972). The effects of meperidine and morphine in rabbits pretreated with phenelzine. *Toxicol. appl. Pharmac.*, **22**, 231–240.

SJÖQVIST, J. C. (1965). Psychotropic drugs (2). Interaction between monoamine oxidase (MAO) inhibitors and other substances. *Proc. Roy. Soc. Med.*, **58**, 967–978.

SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Blockade of endogenous norepinephrine synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **147**, 86–95.

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