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THE INTERRELATION OF HYPOTHERMIA AND DEPLETION OF NORADRENALINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE FROM BRAIN BY RESERPINE, *p*-CHLOROPHENYLALANINE AND α-METHYLMETATYROSINE

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It was shown in 1955 by Pletscher, Shore & Brodie that the sedative and autonomic effects of reserpine in animals were associated with the depletion of 5-hydroxytryptamine (5-HT) and catecholamines from central and peripheral stores. Because of its lack of specificity reserpine is an unsatisfactory tool for distinguishing between the effects of depletion of noradrenaline (NA) and dopamine (DA) on the one hand and 5-HT on the other. Agents are now available which will deplete either catecholamines or 5-HT specifically and we have compared the effects of these depleting agents on body temperature with that of reserpine.

The drugs which we have used for this purpose are *p*-chlorophenylalanine, an inhibitor of tryptophan hydroxylase, which has been shown by Koe & Weissman (1966) to deplete the brain 5-HT of rats to less than 10% of normal without appreciably affecting the catecholamine concentrations, and α-methyl-*m*-tyrosine (α-MMT), which depletes NA and DA without lowering 5-HT. The time course of the action of α-MMT is such that at 4 hr depletion of both NA and DA is maximal, while at 17 hr NA is still lowered and recovery of DA is almost complete (Moore, 1966).

A preliminary account of this work was given at the British Pharmacological Society meeting in Nottingham on January 6, 1967.

METHODS

p-Chlorophenylalanine (p-Cl-Phe) was kindly supplied by Pfizer Ltd. Other drugs were obtained commercially. p-Cl-Phe and α-MMT were injected as ball-milled suspensions or as solutions prepared by dissolving the amino acid in N/1 sodium hydroxide and adding N/10 HCl to give a solution of pH 6–7. p-Cl-Phe was given at the rate of 150 mg/kg intraperitoneally twice daily for 3 days. This regime was adopted since the single dose of 316 mg/kg used by Koe & Weissman (1966) was found to be much less active in mice than in rats. α-MMT was given at the rate of 100 mg/kg intraperitoneally 4 or 17 hr before reserpine. The hydrochloride of phenoxybenzamine, propranolol (Inderal), guanethidine sulphate and bretylium tosylate were dosed at the rate of 30 mg base/kg subcutaneously.

Groups of 8–20 specific pathogen-free mice from the Alderley Park Breeding Unit were used for each temperature measurement. Four mice were used for NA and DA estimations and another four for 5-HT estimations. The brains were pooled in pairs and duplicate estimations were carried out on each pair.

Gastric temperatures were recorded using an orally-inserted probe and displayed on an electrical thermometer type 3GID (Light Laboratories, Brighton, Sussex) calibrated at 0.1° intervals which could be read to 0.05° C. Temperatures were recorded before, and at intervals up to 4 hr after, giving reserpine phosphate (2 mg base/kg). All temperature measurements were carried out in a room with a controlled temperature of 21° C±0.5° C.

The methods used for estimating NA and DA have been described by Chang (1964). 5-HT was extracted into acid butanol, washed with salt saturated pH 10 borate buffer to remove interfering substances, back extracted into 3N HCl and its fluorescence read at a wavelength of 535 mμ when activated with ultraviolet light of 285 mμ wavelength. The more sensitive ninhydrin condensation method for 5-HT (Snyder, Axelrod & Zweig, 1965) was found to be unsuitable owing to interference from *p*-chlorophenylalanine.

RESULTS

The effects of specific depletion of 5-hydroxytryptamine

Figure 1 shows the effects of *p*-chlorophenylalanine on body temperature and brain 5-HT level in mice. NA and DA levels were also measured before and after *p*-chlorophenylalanine treatment and were not significantly altered. It can be seen that,

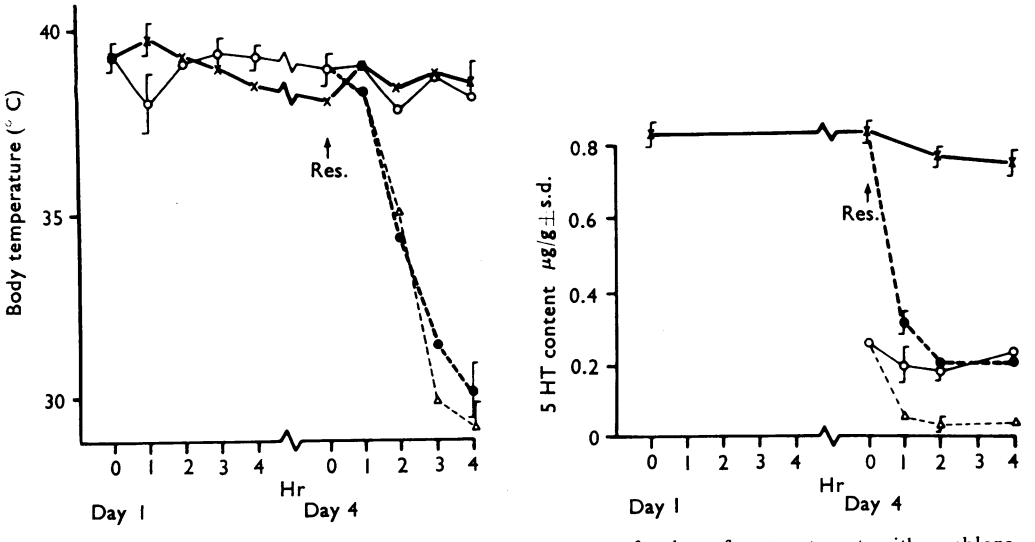

Fig. 1. Mean body temperature and brain 5-HT content of mice after treatment with *p*-chlorophenylalanine (p-Cl-Phe) (○——○) or saline (×——×). The abscissae show the time in hours after the first of 6 doses of p-Cl-Phe (150 mg/kg intraperitoneally twice daily for 3 days), and time after treatment with reserpine, shown by the arrow, on day 4. On day 4 half of the controls (●- - -●) and half of the p-Cl-Phe-treated mice (Δ- - -Δ) received 2 mg base/kg reserpine intraperitoneally. The ordinates indicate mean brain 5-HT content of groups of 4 mice (±S.D.) and mean body temperature (±S.D.) of groups of not less than 8 mice. All experiments were carried out in a temperature controlled room at 21° C±0.5° C. (For clarity, some estimates of standard deviations have been omitted on all diagrams.)

although *p*-chlorophenylalanine lowered 5-HT to the same level as that produced by reserpine, no hypothermia resulted, and that while the combination of the two drugs reduced 5-HT still further the potentiation of reserpine hypothermia was not significant either in respect of the total temperature drop or the rate of attaining it.

The effects of specific depletion of catecholamines

Figure 2 shows the effects of 4-hr pre-treatment with α-MMT on brain NA, DA and body temperature. Measurement of brain 5-HT 4 hr after the administration of α-MMT showed no significant difference between the concentrations in treated and control animals. The catecholamines were reduced by α-MMT to the same level as that produced by reserpine, or lower, but there was no hypothermia or potentiation of reserpine hypo-

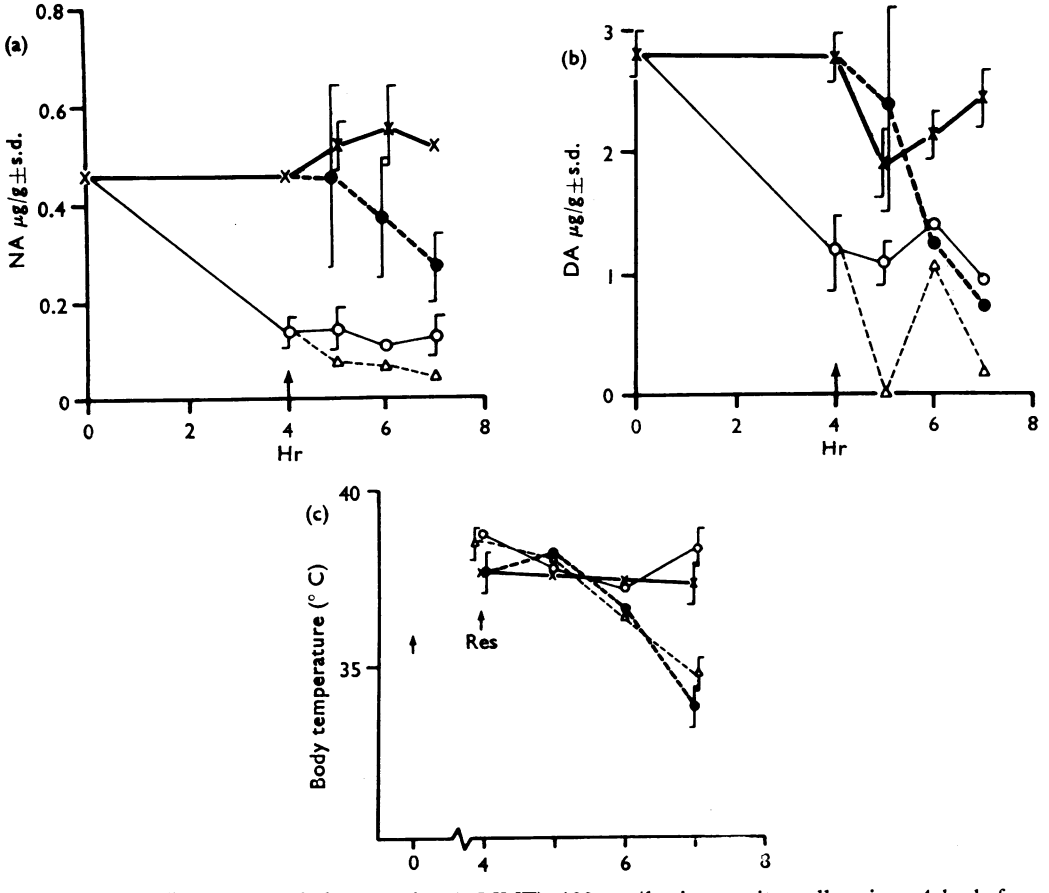

Fig. 2. The effect of α-methyl-*m*-tyrosine (α-MMT), 100 mg/kg intraperitoneally, given 4 hr before reserpine, 2 mg base/kg intraperitoneally, on brain levels of (a) noradrenaline (NA) and (b) dopamine (DA) and on mean body temperature (c). Saline controls (×——×), α-MMT (○——○). At the arrow half the mice in these groups were given reserpine: saline and reserpine (●- - -●), α-MMT + reserpine (Δ- - -Δ). Ordinates indicate mean brain NA or DA content (±S.D.) of groups of 4 mice, and mean body temperature (±S.D.) of groups of 8–16 mice.

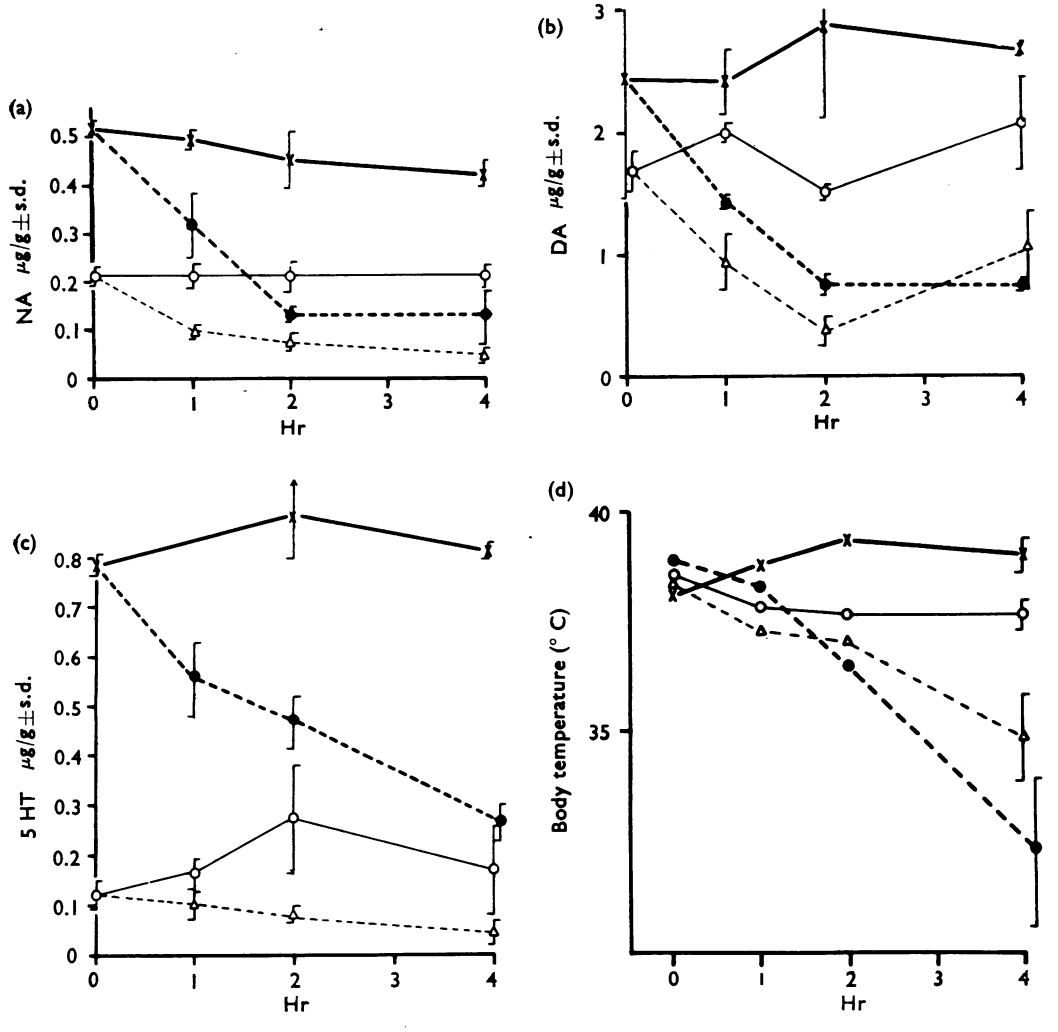

Fig. 3. The effect of combined treatment with p-Cl-Phe and α-MMT on brain levels of NA (a), DA (b), and 5-HT (c), and on body temperature (d). Mice were treated for 3 days with 150 mg/kg of p-Cl-Phe twice daily. On day 4 controls (×——×) received saline, p-Cl-Phe-treated animals received 100 mg/kg intraperitoneally of α-MMT (○——○). Four hours later half of the animals in these groups received 2 mg base/kg reserpine; controls and reserpine (●- - -●) and p-Cl-Phe/α-MMT and reserpine (Δ- - -Δ). The abscissae show time in hours after the dose of reserpine. The ordinates indicate mean brain content of NA, DA and 5-HT (±S.D.) and mean body temperature (±S.D.). Two pairs of mice were used for DA and NA estimations and another two pairs for 5-HT estimations. Temperature was measured in groups of 8–16 mice.

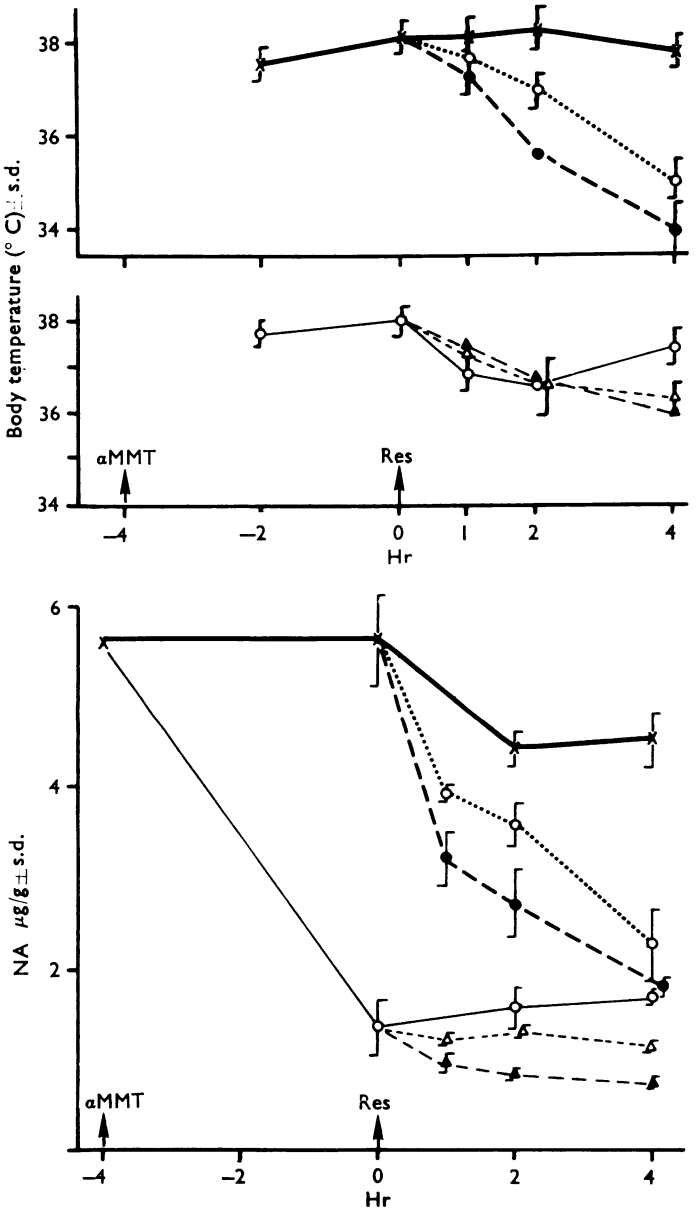

Fig. 4. The effect of submaximal doses of reserpine and α-MMT on brain NA and body temperature. Groups of 48 animals were treated with saline (×——×) or αMMT 100 mg/kg intraperitoneally (○——○). Four hours later, at the arrow, reserpine was given to control mice (○- - -○ = 0.8 mg/kg; ●- - -● = 1 mg/kg), and to α-MMT-treated mice (Δ- - -Δ = 0.8 mg/kg; ▲- - -▲ = 1 mg/kg). The ordinates indicate mean brain concentration of NA (±S.D.) of 4 mice, and mean body temperature (±S.D.) of groups of 8–16 mice.

thermia. The effects of 17-hr pre-treatment with α-MMT were similar to those shown for the 4-hr pre-treatment experiment (Fig. 2). Seventeen hours after α-MMT, 5-HT concentrations were unaltered and NA concentrations were still lowered. Neither hypothermia nor potentiation of reserpine hypothermia was observed due to α-MMT.

Effects of depletion of both noradrenaline and 5-hydroxytryptamine

Figure 3 presents the results of an experiment in which these two regimes were combined, resulting in depletion of both 5-HT and NA and, to a lesser extent, of DA. Although the combination of two drugs produced some fall in temperature compared with controls, it was less than that produced by reserpine, and reserpine-induced hypothermia was not potentiated.

The dose of 2 mg/kg reserpine used in the preceding experiments produced rapid depletion of catecholamines. If under these conditions reserpine caused maximal depletion of catecholamines it would not be possible to detect potentiation of reserpine-induced hypothermia produced by α-MMT. The effects of lower doses of reserpine, which give a slower rate of depletion, in combination with α-MMT were therefore examined and results are shown in Fig. 4.

Mice treated with 0.8 and 1 mg/kg reserpine showed a fall in temperature and a decrease in brain noradrenaline levels. Both of these responses were dose-dependent and when compared with the corresponding curves in Fig. 2 are seen to be submaximal. Treatment with α-MMT produced a reduction in brain NA level to about 37% of the final control level and this was further reduced to 25% and 17% by doses of 0.8 and 1 mg/kg reserpine respectively.

Temperatures of reserpine-treated mice fell progressively after doses of 0.8 and 1 mg/kg. Four hours later mean temperatures were 3° and 4° C lower than controls. In α-MMT-treated mice there was a decrease in temperature of up to 1.4° C at 1 and 2 hr but at 4 hr the mean temperature was not significantly lower than that of the control group. Discrimination between doses of 0.8 and 1 mg/kg reserpine in the hypothermia test was poor in α-MMT-treated mice, and the mean decreases in temperature at 4 hr were 1.6° and 2° C respectively. Thus α-MMT-treatment does not potentiate but opposes the hypothermic effect of submaximal doses of reserpine.

The effects of sympathetic receptor blocking agents

Since it appeared probable from the foregoing experiments that the hypothermia produced by reserpine is not dependent on central depletion of monoamines, some other agents which are known to antagonize the effects of catecholamines at peripheral receptors, or prevent their release from nerve endings have been examined.

Figures 5 and 6 show that phenoxybenzamine by itself produced a fall in temperature greater than that due to reserpine and potentiated reserpine hypothermia while propranolol did not. Neither drug affected monoamine levels, except that phenoxybenzamine reduced the fall in 5-HT produced by reserpine. The actions of bretylium and guanethidine are shown in Fig. 7. Again, neither drug affects amine levels but guanethidine by itself produces some hypothermia and potentiates the hypothermia due to reserpine.

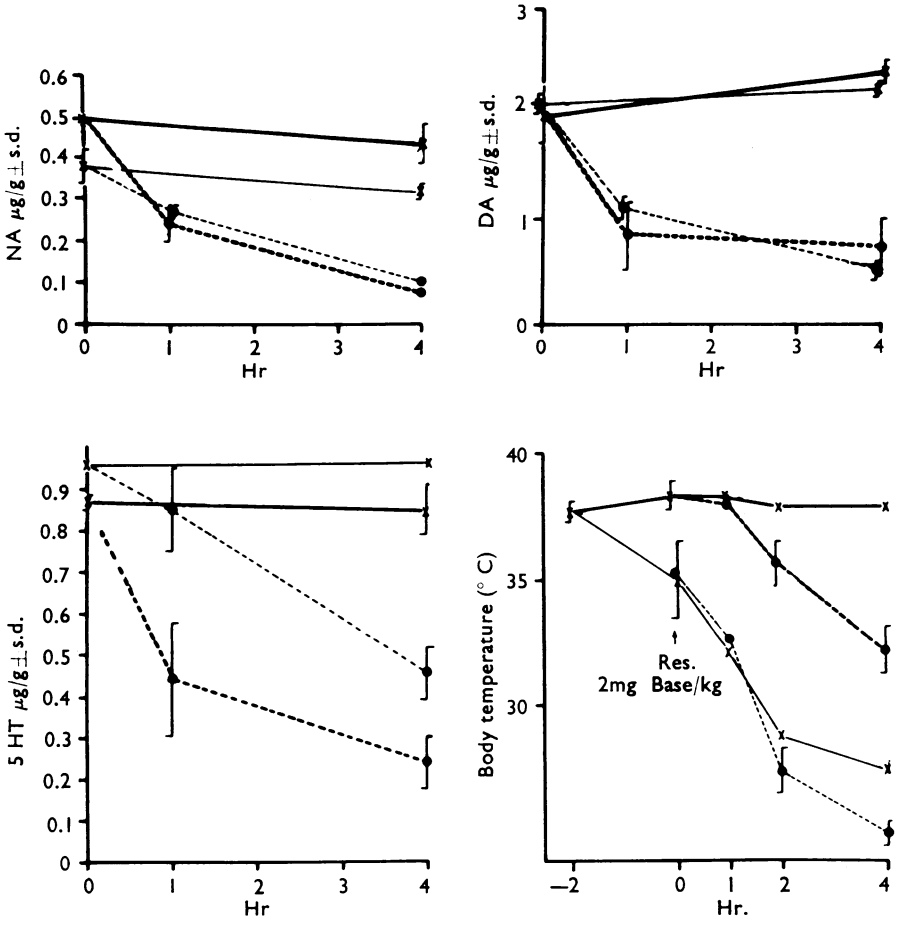

Fig. 5. The body temperature and brain monoamine content of mice treated with phenoxybenzamine and reserpine. Mice were given saline (×——×), or 30 mg/kg phenoxybenzamine subcutaneously (×·····×). Two hours later, at the arrow, half of each group received 2 mg base/kg reserpine: controls and reserpine ●---●, phenoxybenzamine and reserpine ●– – –●. The ordinates indicate mean brain (a) NA, (b) DA or (c) 5-HT (±S.D.), and (d) mean body temperature (±S.D.). Two pairs of mice were used for NA and DA estimations and another two pairs for 5-HT estimations. Temperature was measured in groups of 8–16 mice.

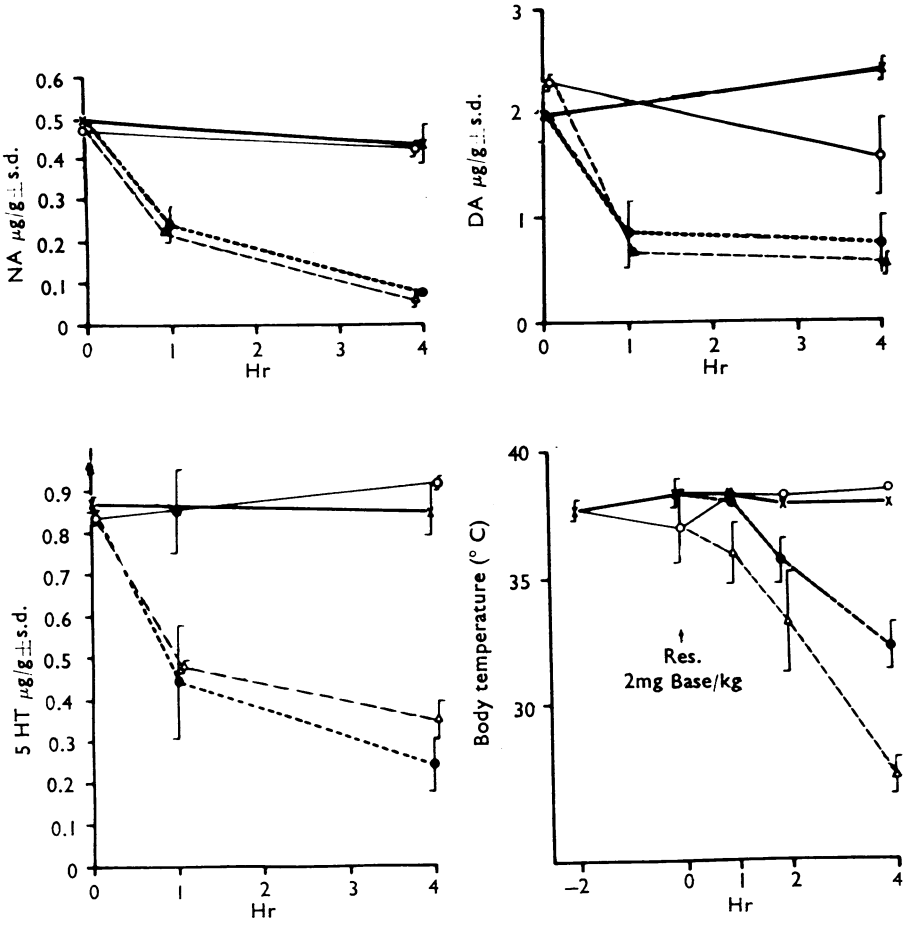

Fig. 6. The body temperature and brain monoamine content of mice treated with propranolol and reserpine (same controls as Fig. 5). Mice were given saline (×——×) or 30 mg/kg subcutaneously propranolol (○——○). Two hours later, at the arrow, half of each group received 2 mg base/kg reserpine: controls and reserpine (●- - -●), propranolol and reserpine (Δ- - -Δ). The ordinates indicate mean brain (a) NA, (b) DA or (c) 5-HT (±S.D.), and (d) mean body temperature (±S.D.). Two pairs of mice were used for NA and DA estimations and another two pairs for 5-HT estimations. Temperature was measured in groups of 8–16 mice.

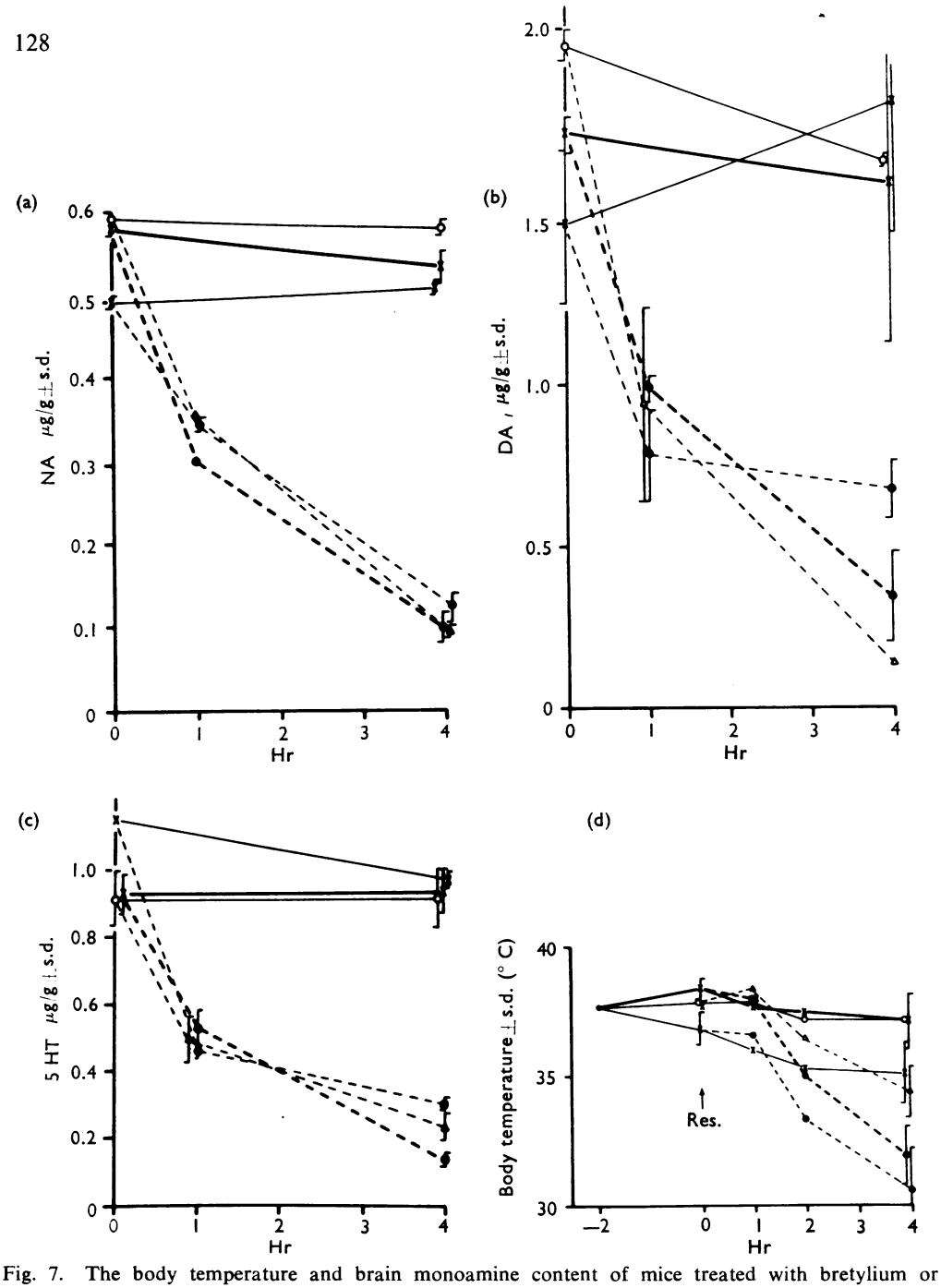

Fig. 7. The body temperature and brain monoamine content of mice treated with bretylium or guanethidine and reserpine. Mice received saline (×——×), bretylium 30 mg/kg subcutaneously (●---○) or guanethidine (×——×). Two hours later half of each group received 2 mg base/kg reserpine. Controls and reserpine (●----●), bretylium and reserpine (Δ- - -Δ), guanethidine and reserpine (●– – –●). The ordinates indicate mean brain (a) NA, (b) DA and (c) 5-HT (±S.D.), and (d) mean body temperature (±S.D.). Two pairs of mice were used for NA and DA estimations and another two pairs for 5-HT estimations. Temperature was measured in groups of 8–16 mice.

DISCUSSION

Whether the sedative and hypothermic effects of reserpine are mediated by 5-HT or by catecholamines has long been the subject of controversy. Brodie, Comer, Costa & Dlabac (1966) consider that 5-HT is the more significant factor and that symptoms correlate better with the initial rate of release rather than with the final extent of depletion. In their view reserpine blocks the membrane pump which is responsible for the uptake of liberated amines from the extracellular fluid. This results in a rapid release of amine and a saturation of the receptors, which persists due to constant replenishment by newly synthesized amine until the membrane pump is restored to normal functioning. Our failure to produce the symptoms of reserpine by specific depletion of 5-HT could be accounted for on this theory, since *p*-chlorophenylalanine is an inhibitor of 5-HT synthesis and therefore would be expected to produce a slow depletion without saturation of the receptors. However, there are several objections to Brodie's mechanism: firstly, desmethylimipramine is believed to inhibit the " membrane pump " and should therefore release amines and potentiate the effects of reserpine, which it does not. Secondly, there is no obvious reason why the same mechanism should not be applied to noradrenaline and dopamine with resulting saturation of the appropriate receptors. This would produce a peripheral stimulation which is not in fact observed. It seems to us simpler to assume that release of 5-HT is relatively unimportant to the hypothermic action of reserpine in mice.

Carlsson (1966) ascribes the major role in the action of reserpine to a block of noradrenaline uptake by intraneuronal storage particles. He also considers that the functionally essential pool of amine must be very small and that considerable depletion of non-specific sites may take place without affecting it. While it is always arguable that a very small, highly specific pool of this sort may exist, the fact that α-MMT can produce a much greater depletion of noradrenaline than reserpine without lowering temperature (Fig. 2) and that the depletion due to submaximal doses of reserpine is greatly enhanced by α-MMT without corresponding enhancement of hypothermia seems to be strong evidence against it. α-MMT has little effect on body temperature and in Fig. 5 it can be seen that as far as hypothermia is concerned there is some antagonism between the two drugs.

There have been many attempts to relate brain concentrations of monoamines to temperature regulation and it is assumed that integrity of monoamine stores is associated with maintenance of body temperature. Cooper (1966) has reviewed evidence concerning the role of hypothalamic amines in the control of body temperature. The evidence for the importance of either NA or 5-HT is based on the alteration of body temperature by intraventricular injections, a route which is assumed to bypass the blood-brain barrier. In cats, injection of 5-HT into the lateral ventricles (Feldberg & Myers, 1963, 1964, 1965) caused a rise in body temperature and noradrenaline caused a fall. Similar responses were found in the dog and monkey. Infusion of 5-HT caused a fall and noradrenaline a rise in body temperature in the rabbit (Cooper, Cranston & Honour, 1965), and in the sheep (Bligh, 1966). In the goat (Anderssen, Jobin & Olsson, 1966) 5-HT caused a fall in temperature, while noradrenaline had no effect. In the mouse intracranial injection of noradrenaline produces a dose-dependent fall in temperature (Brittain, 1966), and 5-HT also produces a fall, so it is difficult to decide which of the amines is the more

important and whether either has a tonic or inhibitory role. The results of our study do not support a central role of either catecholamines or 5-HT in temperature regulation in the mouse.

Phenoxybenzamine presumably acts by reducing the tone of the peripheral blood vessels through blockade of sympathetic α-receptors, resulting in vasodilatation and increased heat loss. Propranolol has no action on α-receptors but it is known to inhibit the stimulatory effects of adrenaline on free fatty acid mobilization and glycolysis, which lead to heat production (Marshall, Barnes, Beane, Maiolo & Schwab, 1965). The fact that propranolol does not produce hypothermia while phenoxybenzamine does, suggests that compensating mechanisms can deal more effectively with variations in heat production than with alterations in heat conservation. Bretylium and guanethidine appear to block adrenergic nerves without affecting receptors: the action of NA on receptors is, in fact, potentiated by these drugs. In addition, guanethidine causes some depletion of peripheral NA. Neither penetrates the brain to any extent and central amine levels are unaffected. In view of their known hypotensive action, it might have been expected that they would increase heat loss and produce hypothermia. In fact guanethidine produces only a slight fall in temperature with a correspondingly slight potentiation of reserpine hypothermia, while bretylium does neither. This suggests that more than adrenergic neurone blockade is involved in the hypothermic action of reserpine.

The hypothermic effects of reserpine do not appear to be dependent on central depletion of 5-HT, NA or DA, and, although hypothermia can be produced by peripheral α-adrenergic blockade, it is not produced by either depletion of NA or blockade of the adrenergic nerve terminal.

The possibility remains that reserpine may have both central and peripheral actions in causing hypothermia but that in these experiments the peripheral outweighs the central component.

SUMMARY

1. Reserpine (2 mg/kg intraperitoneally) reduces brain levels of noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) in the mouse and simultaneously lowers body temperature.

2. *p*-Chlorophenylalanine (150 mg/kg intraperitoneally twice daily for 3 days) lowers brain 5-HT to the same extent as reserpine (2 mg/kg intraperitoneally) without producing hypothermia or affecting the extent or course of the hypothermia produced by a subsequent dose of reserpine.

3. α-Methyl-*m*-tyrosine (100 mg/kg intraperitoneally) lowers brain NA and DA to the same extent as reserpine (2 mg/kg intraperitoneally) without producing hypothermia or affecting the hypothermia caused by reserpine, and it potentiates the depletion produced by smaller doses of reserpine (0.8 to 1 mg/kg).

4. The combination of *p*-chlorophenylalanine and α-methyl-*m*-tyrosine is similarly without significant effect on temperature.

5. Phenoxybenzamine (30 mg/kg subcutaneously) produces a marked hypothermic effect, while propranolol (30 mg/kg subcutaneously) does not.

6. Guanethidine (30 mg/kg subcutaneously) produces a slight hypothermia, while bretylium (30 mg/kg subcutaneously) does not.

7. It is concluded that the control of body temperature is not necessarily linked with brain levels of NA, DA or 5-HT and that the hypothermic action of reserpine may be peripheral rather than central.

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REFERENCES

ANDERSSON, B., JOBIN, M. & OLSSON, K. (1966). Serotonine and temperature control. *Acta physiol. scand.*, **67**, 50–56.

BLIGH, J. (1966). Effects on temperature of monoamines injected into the lateral ventricles of sheep. *J. Physiol., Lond.*, **185**, 46P–47P.

BRITTAIN, R. T. (1966). The intracerebral effects of noradrenaline and its modification by drugs in the mouse. *J. Pharm. Pharmac.*, **18**, 621–623.

BRODIE, B. B., COMER, M. S., COSTA, E. & DLABAC, A. (1966). The role of brain serotonin in the mechanism of the central action of reserpine. *J. Pharmac. exp. Ther.*, **152**, 340–349.

CARLSSON, A. (1966). Pharmacological depletion of catecholamine stores. *Pharmac. Rev.*, **18**, 541–549.

CHANG, C. C. (1964). A sensitive method for spectrophotofluorimetric assay of catecholamines. *Int. J. Neuropharmac.*, **3**, 643–649.

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

COOPER, K. E. (1966). Temperature regulation and the hypothalamus. *Br. med. Bull.*, **22**, 238–242.

FELDBERG, W. & MYERS, R. D. (1963). A new concept of temperature regulation by amines in the hypothalamus. *Nature, Lond.*, **200**, 1325.

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.

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

MARSHALL, R. J., BARNES, W. E., BEANE, J. E., MAIOLO, J. A. & SCHWAB, L. T. (1965). Blockade by Propranolol (I.C.I. 45,520) of the hemodynamic and metabolic responses to infused catecholamines. *Fedn Proc.*, **24**, 713.

MOORE, K. E. (1966). Effects of α-methyltyrosine on brain catecholamines and conditioned behavior in guinea pigs. *Life Sci. Oxford*, **5**, 55–65.

PLETSCHER, A., SHORE, P. A. & BRODIE, B. B. (1955). Serotonin release as a possible mechanism of reserpine action. *Science, N.Y.*, **122**, 374–375.

SNYDER, S. H., AXELROD, J. & ZWEIG, M. (1965). A sensitive and specific fluorescence assay for tissue serotonin. *Biochem. Pharmac.*, **14**, 831–835.