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THE EFFECT OF AMBIENT TEMPERATURE ON THE ACTIONS OF TREMORINE ON BODY TEMPERATURE AND ON THE CONCENTRATION OF NORADRENALINE, DOPAMINE, 5-HYDROXYTRYPTAMINE AND ACETYLCHOLINE IN RAT BRAIN

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Tremorine (1,4-dipyrrolidino-2-butyne) was first introduced by Everett, Blockus & Shepperd in 1956. Since that time there have been reports of its effects on brain acetylcholine (Pepeu, 1963; Holmstedt & Lundgren, 1965) and brain dopamine (Everett, 1964; Holmstedt & Lundgren, 1965). Reports of its effects on brain 5-hydroxytryptamine (5-HT) and noradrenaline have varied. Friedman, Aylesworth & Friedman (1963) demonstrated a decrease in the amount of noradrenaline in rat brain and an increase in the amount of 5-HT, whereas Whittaker & Walaszek (1964) using the same species could not detect changes in either of these substances. Also Holmstedt & Lundgren (1965) could not detect any change in rat brain noradrenaline, but Everett (1964) reported a significant decrease.

Friedman & Everett (1964) suggested that the increase in brain 5-HT could be related to tremorine's hypothermic action. Tremorine reduced the body temperature of rats in doses which induced tremor (Ferrari & Gessa, 1964).

It is possible that the different effects reported for tremorine on brain 5-HT are related to different ambient temperature conditions, particularly as no mention of ambient temperature is made in the conflicting reports.

The object of this investigation was to study the effect of ambient temperature on the action of tremorine both on body temperature and on the amounts of noradrenaline, dopamine, 5-HT and acetylcholine in rat brain.

METHODS

Male Wistar rats weighing 200 to 250 g were used in all the experiments. Drug injections were made by the intraperitoneal route, and the animals were placed in the selected ambient temperature immediately after injection.

Brain amine estimation

Whole brain noradrenaline, dopamine and 5-HT were assayed by a fluorimetric method. A 0.4

N-perchloric acid extract of 4 rat brains was neutralized to pH 6.5 (Bertler, Carlsson & Rosengren, 1958). The neutralized extract was passed over a Dowex resin column (50 W-X4, 200–400 mesh) prepared in the sodium form (Wiegand & Perry, 1961). Noradrenaline and dopamine were eluted with 1M-potassium chloride solution buffered at pH 6.5 and separate fluorophores prepared by different oxidation procedures (Carlsson & Waldeck, 1958; Bertler *et al.*, 1958), 5-HT was then eluted with 15 ml. 0.1 N-sodium hydroxide solution containing 0.2% EDTA into 1.5 ml. 5N-sodium acetate buffer pH 4.6.

Fluorimetric readings were made using a Farrand Spectrophotofluorimeter. Activation and fluorescence wavelengths used were: noradrenaline (fluorophore) 394/500 mμ, dopamine (fluorophore) 335/380 mμ, and 5-HT (read direct) 292/342 mμ. Estimations were made using brains from rats pretreated with normal saline and tremorine. Ambient temperatures of 6, 16, 26 and 36° C were used during the study.

Brain acetylcholine estimation

Acetylcholine was extracted from single rat brains by the method of MacIntosh & Perry (1950). A 16-point assay was conducted on guinea-pig ileum pretreated for 30 min with Krebs solution containing mipafox (N, N^1 di-iso-propylphosphorodiamidic fluoride), 100 μg/ml. The ileum was suspended in Krebs solution containing morphine 100 μg/ml. (Paton, 1957), and the doses added according to a Latin Square design.

After the assay a sample of extract was boiled for 1 min with 1N-sodium hydroxide, neutralized with 1N-hydrochloric acid and an aliquot tested for activity. Another sample of extract was tested against standard acetylcholine on the same ileum preparation which had been pretreated with a low dose of atropine. The results were discounted from the small number of extracts which showed either activity after boiling, or, when compared with standard acetylcholine, an unequal reduction in contraction height after atropine. Estimations were made using brains from rats pretreated with normal saline and tremorine. Ambient temperatures of 6, 26 and 36° C were used during the study.

Internal standards were included in both brain amine and brain acetylcholine estimations. The values reported in μg/g whole brain ± standard error were corrected for recoveries. Average recoveries were noradrenaline 95%, dopamine 85%, 5-HT 100%, and acetylcholine 78%.

Rectal temperature

Rat rectal temperatures were measured using copper-constantan thermocouples inserted to a depth of 4 cm. The rats were placed in the selected ambient temperatures (6, 16, 26 and 36° C) and rectal temperatures recorded at 5 min intervals for 90 min after pretreatment with either normal saline or tremorine.

Materials

Tremorine (1, 4-dipyrrolidino-2-butyne) as the hydrochloride.

L-noradrenaline bitartrate, serotonin creatinine sulphate (5-hydroxytryptamine), 3-hydroxytyramine hydrochloride (dopamine), mipafox (N, N^1 di-iso-propyl-phosphorodiamidic fluoride), (Koch-Light Laboratories Limited).

Acetylcholine chloride (British Drug Houses Limited). All doses and concentrations refer to free base.

RESULTS

Brain amines

1. *Ambient temperature 26° C.* The effect of tremorine 20 mg/kg on the concentrations of dopamine, 5-HT, and noradrenaline in the brains of rats maintained at an ambient temperature of 26° C ± 0.5° C is shown in Fig. 1. Control values were estimated 15, 30, 60 and 90 min after saline injection and were not significantly different ($P > 0.05$)

from each other. Therefore these results were grouped to give a single mean control value. No significant change occurred in the amounts of dopamine or 5-HT in brain at any time after injection of tremorine 20 mg/kg. A decrease in the amount of noradren-

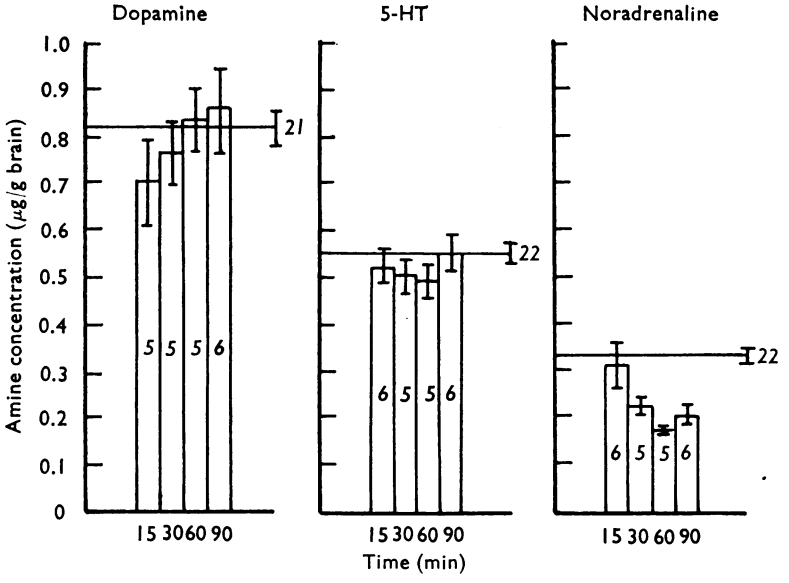

Fig. 1. The concentration of dopamine, 5-HT and noradrenaline (μg/g whole brain ± standard error) in the brains of rats maintained at 26° C. Horizontal lines represent mean values from rats pretreated with saline. Open columns represent mean values from rats pretreated with tremorine 20 mg/kg for the times indicated. Figures in italics indicate the number of observations for each mean value.

aline in brain was observed which was significant ($P<0.05$) at 30 min after tremorine injection, maximal at 60 min and still present at 90 min.

2. *Ambient temperature 16° C.* The effect of three doses of tremorine (5, 10 and 20 mg/kg) on the concentrations of dopamine, 5-HT and noradrenaline in the brains of rats maintained at an ambient temperature of 16° C ± 0.5° C is shown in Fig. 2. Estimations were made 60 min after injecting the rats and placing them in an ambient temperature of 16° C.

Control values at this temperature did not differ significantly from control values at 26° C. There was no significant change in the amount of dopamine in brain with any of the doses of tremorine. With the 5 mg/kg dose of tremorine there was no significant change in either brain noradrenaline or 5-HT. With the higher doses a significant decrease in brain noradrenaline and a significant increase in brain 5-HT was observed.

3. *Ambient temperature 6° C.* The effect of tremorine 5 mg/kg and 20 mg/kg on brain amines at an ambient temperature of 6° C ± 0.5° C is shown in Fig. 3. There was no significant change in brain dopamine 60 min after injection when compared with control values. The amount of noradrenaline in brain was significantly decreased with

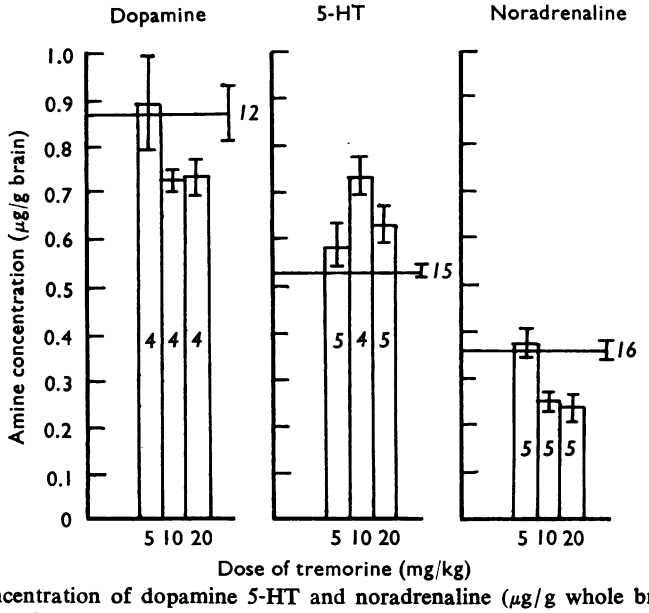

Fig. 2. The concentration of dopamine 5-HT and noradrenaline (μg/g whole brain ± standard error) in the brains of rats maintained at 16° C for 60 min after injection. Horizontal lines represent mean values from rats pretreated with saline. Open columns represent mean values from rats pretreated with tremorine in the dose indicated. Figures in italics indicate the number of observations made for each mean value.

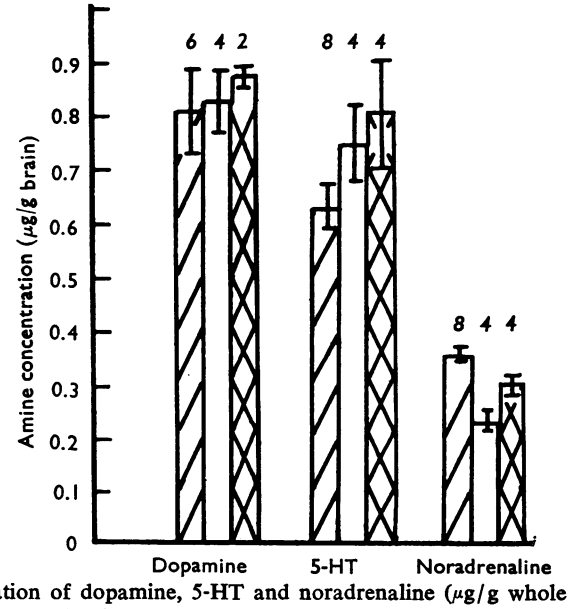

Fig. 3. The concentration of dopamine, 5-HT and noradrenaline (μg/g whole brain ± standard error) in the brains of rats maintained at 6° C for 60 min after injection. Hatched columns represent mean values from rats pretreated with saline. Open columns represent mean values from rats pretreated with tremorine 20 mg/kg. Cross-hatched columns represent mean values from rats pretreated with tremorine 5 mg/kg. Figures in italics indicate the number of observations for each mean value.

the 20 mg/kg dose but unchanged after the 5 mg/kg dose. Neither control values at this temperature nor the 5 mg/kg tremorine values differed significantly from control values at 16° C and 26° C. In both of the tremorine pretreated groups the amount of 5-HT in brain was significantly higher than control values at 16° C and 26° C, and tremorine pretreated values at 26° C. However, they were not significantly higher ($P>0.05$) than their own control values at 6° C.

4. *Ambient temperature 36° C.* The effect of tremorine 20 mg/kg on brain amines at 36° C was measured 45 min after administration. Some animals died at a time between 45 and 60 min. The results are shown in Fig. 4. The amount of dopamine under these

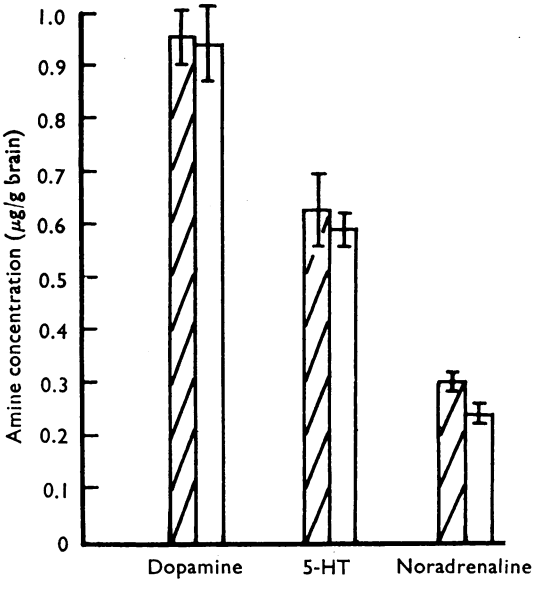

Fig. 4. The concentration of dopamine, 5-HT and noradrenaline (μg/g whole brain ± standard error) in the brains of rats maintained at 36° C for 45 min after injection. Hatched columns represent mean values from rats pretreated with saline. Open columns represent mean values from rats pretreated with tremorine 20 mg/kg. All values represent the mean of 4 determinations.

conditions was not significantly different from the amount in control animals. Brain 5-HT was also unchanged after tremorine, but brain noradrenaline was significantly decreased.

Brain acetylcholine

1. *Ambient temperature 26° C.* The effect of tremorine 20 mg/kg on brain acetylcholine in rats maintained at 26° C is shown in Fig. 5. The amount of acetylcholine was significantly higher than control values, at 5, 15, 30 and 60 min after injection. The highest value recorded being at 30 min.

2. *Ambient temperature 6° C and 36° C.* When the ambient temperature was reduced to 6° C the mean control value at 30 min was not significantly different from the mean value at 26° C. Rats pretreated with tremorine 20 mg/kg for 30 min at 6° C showed a significant increase in brain acetylcholine over controls at 6° C. This increase was not significantly different from the increase at 26° C. Similarly the control value at 36° C, 30 min after the injection of saline, was not significantly different from the control values at 6° C and 26° C. Also the increase in brain acetylcholine after tremorine at 36° C was not significantly different from the increases at 6° C and 26° C (Fig. 5).

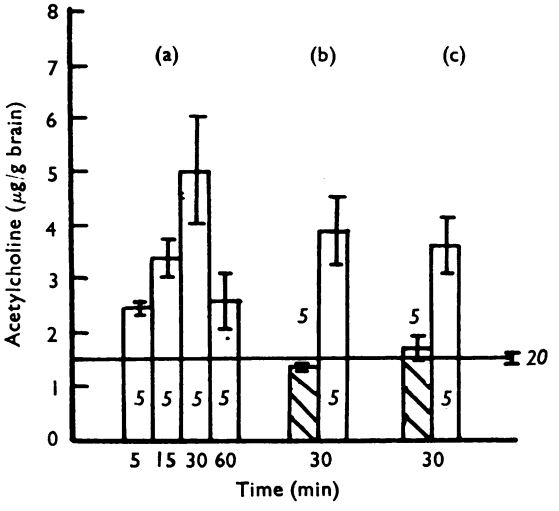

Fig. 5. The concentration of acetylcholine (μg/g whole brain ± standard error) in the brains of rats maintained at (a) 26° C, (b) 6° C and (c) 36° C for the times indicated. The horizontal line and hatched columns represent rats pretreated with saline. Open columns represent rats pretreated with tremorine 20 mg/kg. Figures in italics indicate the number of observations for each mean value.

3. *Rectal temperature.* The rectal temperature of rats maintained at an ambient temperature of 16° C after injection of either saline or tremorine (5, 10 and 20 mg/kg) is shown in Fig. 6. At this ambient temperature the animals pretreated with saline showed a fall in rectal temperature of less than 1° C in 90 min. After tremorine injection there was a pronounced fall in rectal temperature which was dose related. The time after injection at which the rectal temperatures became significantly different from control was 15 min with the 5 mg/kg and 10 mg/kg dose and 5 min with the 20 mg/kg dose.

The rectal temperature of rats injected with saline and exposed to ambient temperatures of 6°, 16°, 26° and 36° C is shown in Fig. 7. At the three lower ambient temperatures, only slight changes in rectal temperature were seen. The lowest rectal temperature recorded was 36.6° C after 90 min at 6° C. The mean rectal temperature of the rats at 36° C showed a progressive increase, the maximum value reached being at 75 min of exposure to this temperature.

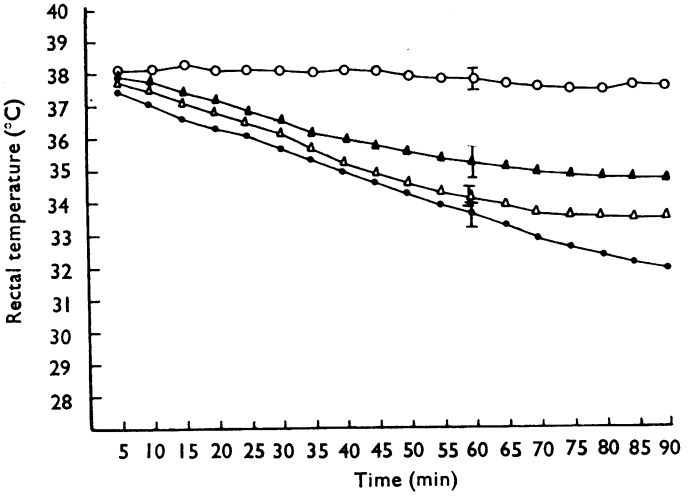

Fig. 6. The effect of tremorine on the rectal temperature of rats maintained at 16° C ambient temperature. ○=saline pretreated controls, ▲=tremorine 5 mg/kg, △=tremorine 10 mg/kg, ● =tremorine 20 mg/kg. Each value represents the mean of at least 11 observations.

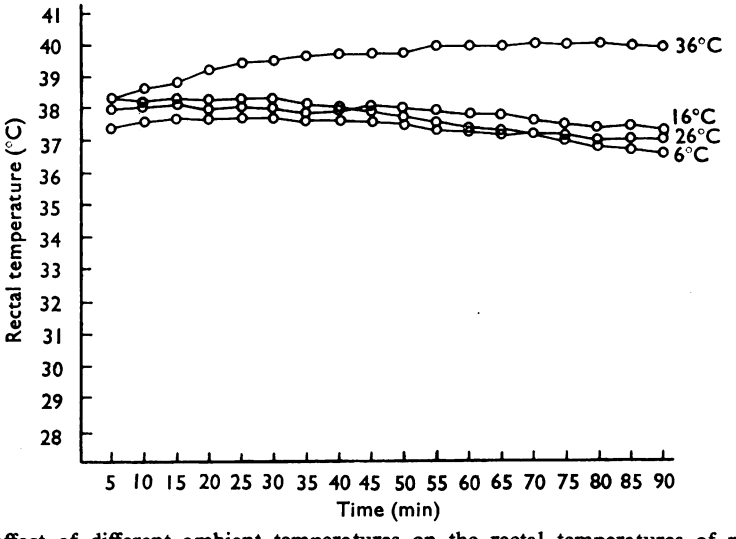

Fig. 7. The effect of different ambient temperatures on the rectal temperatures of rats pretreated with saline (○). Each value represents the mean of at least 11 observations.

The rectal temperature of rats maintained under conditions similar to those in Fig. 7 but receiving tremorine 20 mg/kg is shown in Fig. 8. At 26° C the rectal temperature follows closely the pattern shown by control animals at this temperature. It is not significantly different from controls at any time. The effect of 20 mg/kg tremorine at 16° C has been noted previously (Fig. 6). At 6° C this dose of tremorine produced a marked fall in rectal temperature which was significantly lower than control values after 5 min, and at 90 min had fallen 9.1° C below the mean rectal temperature of the control group.

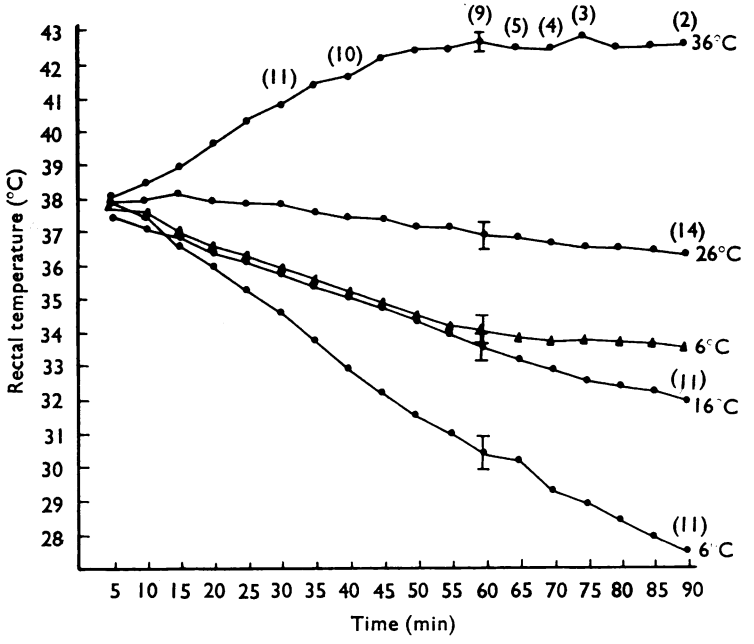

Fig. 8. The effect of different ambient temperatures on the rectal temperatures of rats pretreated with tremorine 5 mg/kg (▲) and 20 mg/kg (●). Numbers in parentheses indicate the number of observations made.

With 20 mg/kg tremorine at 36° C there was a progressive increase in rectal temperature to a time of 60 min. The mean rectal temperature of the treated group was significantly higher ($P<0.05$) than control means after 25 min. Some animals died after tremorine injection at this temperature. At 65 min only 5 out of a group of 11 rats survived and at 90 min there were only 2 survivors.

The effect of tremorine 5 mg/kg on rat rectal temperature at an ambient temperature of 6° C is also shown in Fig. 8. During the first 60 min the fall in rectal temperature followed very closely the fall seen with 20 mg/kg tremorine at 16° C. After 60 min the rate of fall was decreased.

DISCUSSION

Our results show that the effect of tremorine on the concentration of 5-HT in rat brain is dependent on the ambient temperature. At 36° C and 26° C there was no alteration in the amount of 5-HT. At 16° C there was a clear increase, while at 6° C there was suggestive evidence of an increase. These findings may present an explanation for the conflicting reports quoted in the introduction (Friedman *et al.*, 1963; Whittaker & Walaszek, 1964).

It is possible that the increase in 5-HT is related to the hypothermia produced by tremorine. If there was no change in rectal temperature after tremorine, as at 26° C,

then there was also no change in brain 5-HT. If the fall in rectal temperature was less than 3° C, as with 5 mg/kg tremorine at 16° C, then there was also no change in brain 5-HT. If the rectal temperature fell more than 3° C, as with 10 and 20 mg/kg tremorine at 16° C, then there was an increase in brain 5-HT.

Interpretation of the results from the experiments at 6° C is complicated because of the non-significant increase seen in the amount of 5-HT in the brains of control rats. This meant that no significant change could be shown between control and tremorine treated groups at 6° C. However, if the comparison was made between tremorine values at 6° C and controls at 16° C or 26° C or tremorine values at 26° C, then the amount of 5-HT in brain at 6° C showed a significant increase after doses of tremorine of 5 and 20 mg/kg. Both these doses produced a fall in rectal temperature greater than 3° C. It would seem possible therefore that no change in brain 5-HT would be observed after tremorine unless the fall in rectal temperature was 3° C or more. This effect of tremorine shows some similarities to the action of chlorpromazine. A number of workers have suggested that the effects of chlorpromazine on 5-HT metabolism is related to the hypothermia produced (Costa, Gessa & Brodie, 1962 ; Morpugo, 1962 ; Pletscher, Kunz, Staebler & Gey, 1963 ; Bartlet, 1965). These reports are mainly concerned with the ability of chlorpromazine to inhibit the rise in 5-HT seen after monoamine oxidase inhibitors. However, it has also been reported (Bartlet, 1960) that chlorpromazine administered alone gave a significant increase in mouse brain 5-HT 1 to 6 hr after injection. It is unlikely that the increased amount of 5-HT in brain is related to an increased rate of synthesis. Reid, Volicer, Beavan & Brodie (1966) could not detect any change in 5-HT synthesis in brain after cold exposure at 6° C. El Hawary, Feldberg & Lotti (1967) have demonstrated in cats an increase in the concentration of 5-HT in third ventricle perfusate, which was associated with shivering and a rise in body temperature. Therefore, it would seem that cold stress will increase the rate of release of 5-HT at least from certain brain areas. Thus it would seem that the most likely explanation for the temperature-dependent increase in 5-HT after tremorine is a decreased loss from the brain. Reid *et al.* (1966) have shown an increase in the synthesis rate of 5-HT in the brains of rats maintained at an ambient temperature of 38° C. Under similar conditions (45 min at 36° C) we noted an increase in the amount of 5-HT in brain when compared with amount recorded at 26° C. However, the difference in these values was not significant ($P>0.05$).

The ability of tremorine to produce a decrease in the amount of noradrenaline in rat brain was not affected by changes in ambient temperature, and is therefore also independent of changes in rectal temperature. A dose of tremorine greater than 5 mg/kg was required before this effect was observed. The mechanism of action of the depletion is not known, but Anton, Rodriguez & Friedman (1967) have shown that tremorine-induced depletion of cardiac noradrenaline is inhibited by atropine. They therefore suggest that it is a cholinergically mediated response. These workers could not demonstrate any change in brain noradrenaline in these experiments, a result in conflict with our work and with a previous result reported by one of them (Friedman *et al.*, 1963). They suggest this may be a result of strain differences. If this is so, it could account for the conflicting reports on the effect of tremorine on brain noradrenaline.

The ability of tremorine to produce an increase in brain acetylcholine is also independent of ambient temperature and of rectal temperature. This increase was

significant as early as 5 min after tremorine injection at a time when the tremor had developed. This observation confirms the findings of other workers (Holmstedt, Lundgren & Sundwall, 1963; Pepeu, 1963; Holmstedt & Lundgren, 1965). Significant changes in the amounts of noradrenaline and 5-HT in brain occurred only at a later time, usually when the tremor was decreasing in intensity. This lends weight to the postulate that it is the increase in brain acetylcholine which is the causative agent in tremorine-induced tremor. The mechanism of the increase in acetylcholine has not been elucidated, but it does not seem to be an action on either cholineacetylase or cholinesterase (Holmstedt, Lundgren, Schuberth & Sundwall, 1965).

No change in brain dopamine was observed at any time after tremorine with any of the doses used. These results confirm the reports of other workers (Everett, 1964; Holmstedt & Lundgren, 1965).

As would be expected, a decrease in the ambient temperature resulted in an increase in the rate of fall of rectal temperature produced by a given dose of tremorine. When the ambient temperature was increased to 36° C, tremorine produced an increase in rectal temperature significantly greater than the increase measured in control rats. The increase was probably due to the tremor, which would cause heat production. Under these conditions the toxicity of tremorine was markedly increased. At 26° C none of the rats died with the 20 mg/kg dose, while at 36° C only 2 rats out of a group of 11 survived as long as 90 min. These animals died usually when their rectal temperature reached 43° C.

Thus the ambient temperature plays an important part in determining the effects of tremorine in the rat. By maintaining the animals at an ambient temperature of 26° C these variations may be eliminated.

SUMMARY

1. The effect of different ambient temperatures on the actions of tremorine on rectal temperature and on the amounts of noradrenaline, dopamine, 5-HT and acetylcholine in rat whole brain has been studied.

2. Tremorine produced an increase in brain acetylcholine and a decrease in brain noradrenaline; both effects were independent of the ambient temperature.

3. No change in brain dopamine was observed at any temperature.

4. Tremorine-induced increase in brain 5-HT was only seen at ambient temperatures less than 26° C, when the rectal temperature fell 3° C or more.

5. The tremorine-induced hypothermia was greater the lower the ambient temperature.

6. At 36° C tremorine produced an increase in rectal temperature and its toxicity was increased.

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