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The effects of tremorine and oxotremorine in hyperthyroid mice

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Hyperthyroid mice exhibit only transient low-level tremor and hypothermia in response to tremorine and oxotremorine, yet the peripheral effects of these two agents are similar in intensity and duration to those elicited in control animals. These findings support the theory that tremor and hypothermia are both central effects and separate from the peripheral muscarinic actions of tremorine and oxotremorine. Of several possible explanations for the reduction in tremor and hypothermia, the most likely appears to be a potentiation of central adrenergic mechanisms in the hyperthyroid mice.

TREMORINE(1,4-dipyrrolidino-2-butyne) produces both central and peripheral muscarinic effects in laboratory animals (Everett, 1956). These effects are mediated through its active metabolite oxotremorine, (Kocsis & Welch, 1960; George, Haslett & Jenden, 1962). In the mouse, tremorine and oxotremorine cause sustained tremors, miosis, salivation, diarrhoea and hypothermia, all of which can be prevented by centrally-acting anticholinergic drugs (Blockus & Everett, 1957), and to a large extent by centrally-acting adrenergic drugs (Spencer, 1965, 1966; Morpurgo, 1967).

For several reasons, some interaction might be expected between a hyperthyroid state and the effects of tremorine and oxotremorine. A frequent feature of hyperthyroidism in man is a tremor of the hands or of the whole body (Morgans, 1964), which suggests that the tremorgenic effects of both compounds would be enhanced in hyperthyroid animals. On the other hand, treatment with 3,5,3′-triiodothyropropionic acid reduces or prevents the hypothermia produced by chlorpromazine in the rat and ground squirrel (Hoffman, 1959). Potentiation of the cardiovascular and metabolic actions of catecholamines by thyroid hormones has been widely reported (for references see Hess & Shanfeld, 1965); it is possible that thyroid hormones would potentiate catecholamines also at central sites. If this were so, the effects of tremorine and oxotremorine might be antagonized in a way analogous to that produced by the administration of centrally-acting adrenergic drugs such as amphetamine and imipramine (Spencer, 1965, 1966; Morpurgo, 1967).

This report shows that tremorine-induced and oxotremorine-induced hypothermia and tremor are substantially reduced in hyperthyroid mice.

Experimental

Groups of 8 or 10 male adult albino mice of a TO strain, weighing 20 to 25 g, were maintained on water and a 41B cube diet, and kept under constant environmental conditions at 20 to 22°. A maximal level of hyperthyroidism was produced in the mice by the daily subcutaneous

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injection (neck) of thyroxine sodium, 2 mg/kg for 10 days; control animals received the vehicle, 0·01 N sodium hydroxide in 0·9% saline solution, for the same length of time. On the 11th day, 24 hr after the last pre-treatment injection, mice received a subcutaneous injection (flank) of tremorine hydrochloride, 20 mg/kg, or oxotremorine oxalate, 0·5 mg/kg, dissolved immediately before use in 0·9% saline solution. [Preliminary work with this dose of thyroxine had shown that TO mice are maximally hyperthyroid on the 11th day after commencing treatment, using the mouse anoxia test (Spencer & West, 1961) and the oxygen uptake test (MacLagan & Sheahan, 1950) to assess the level of hyperthyroidism].

The effects of tremorine and oxotremorine were determined as follows: *hypothermia* was assessed by measuring oesophageal temperatures of mice before and at intervals after the injection of the compounds, using an oesophageal thermocouple and calibrated electric thermometer (Brittain & Spencer, 1964). Group data have been expressed as the mean $\pm$ s.e., using Student's *t*-test to establish the significance of difference between groups. At the same time, each animal was observed briefly, to assess the intensity of *tremor*, using the following arbitrary scoring system: no tremor = 0, moderate or intermittent tremor = 1, and severe or continuous tremor = 2 marks. Group data have been expressed as the mean score, or "tremor index", and the significance of difference between groups calculated using a modified *t*-test. (The assumption was made that the central limit theorem held, and that the individual variances of different groups were not significantly different from one another). The measurement of oesophageal temperatures and tremor in a group of 10 mice consistently took less than 2 min, this period being spent equally before and after the nominated observation time. In addition, the mice were observed continuously for the presence or absence of peripheral muscarinic effects, particularly salivation and diarrhoea. All of these effects, tremor, hypothermia and the peripheral effects, were observed "blind", the identity of the groups being made known to the operator only at the end of the total observation period.

Results

The production of a hyperthyroid state in mice, using daily injections of thyroxine, did not induce a state of tremor; at no time was a low-level or intermittent tremor observed before the administration of a tremorgenic agent. The oesophageal temperatures of hyperthyroid mice were not significantly different to those of control mice before the administration of tremorine or oxotremorine.

Fig. 1 illustrates the levels of tremor and hypothermia produced by the compounds in control and hyperthyroid mice.

The presence of a hyperthyroid state caused a substantial reduction in the severity and duration of tremorine-induced hypothermia; at each of the three observation times after its injection, the hyperthyroid mice had significantly less hypothermia ($P = <0·001$). Similarly, the tremor induced in hyperthyroid mice by tremorine was more transient and of

lower intensity than in controls (P = <0·05 at 60 min). This reduction in effect of tremorine was not due to an effect of thyroxine on liver enzymes, preventing the *in vivo* appearance of its metabolite, oxotremorine, since the the effects of injected metabolite were also markedly reduced in hyperthyroid mice (Fig. 1). After oxotremorine injection, there was a highly significant reduction in the level of hypothermia at each of the observation times (P = <0·001), and the tremor was almost completely inhibited; only occasional mice exhibited a low-level, intermittent tremor, and this reduction in effect was statistically significant (P = <0·05 at 30 and 60 min). When no compound was administered, a subcutaneous injection of 0·9% saline solution, 0·2 ml/20 g body weight, produced neither tremor nor a change in oesophageal temperatures of either control or hyperthyroid mice.

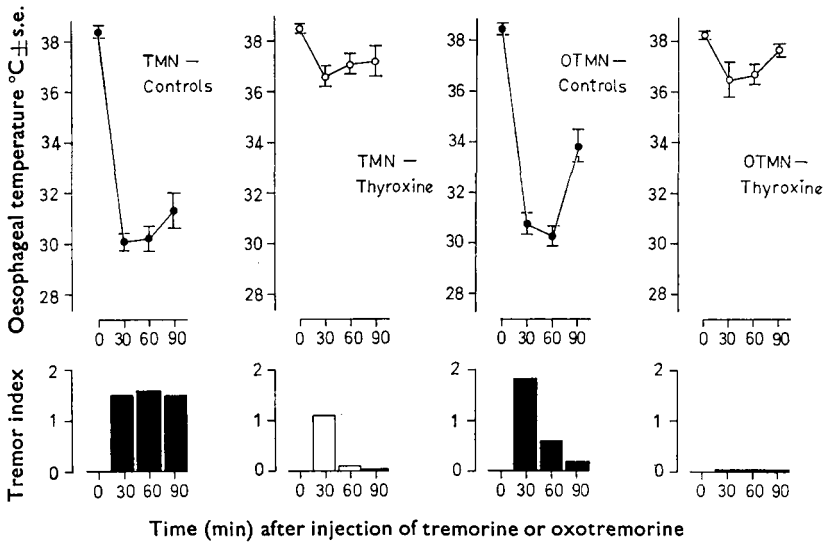

FIG. 1. Tremorine-induced (TMN) and oxotremorine-induced (OTMN) tremor and hypothermia in control and thyroxine-treated mice. Mice were pretreated with thyroxine, (2 mg/kg), or the vehicle, for 10 days; on the 11th day, each mouse received a s.c. injection of tremorine (20 mg/kg) or oxotremorine (0·5 mg/kg).

In contrast to the marked reductions in tremor and hypothermia, the production of a hyperthyroid state had no inhibitory effect upon the intensity and duration of the peripheral muscarinic effects of injections of both compounds, marked salivation and diarrhoea being produced in the hyperthyroid mice and instead of being reduced, these effects occasionally showed evidence of potentiation.

In a final experiment, oxotremorine was injected into control mice kept in an incubator at 30° for 2 hr before, and throughout the experiment. Under these conditions, these mice did not show hypothermia in response to oxotremorine, yet the intensity of tremor was as great as that present in

control mice kept at 22° and given oxotremorine. It was concluded that the absence of hypothermia at 30° in the response to the compounds would not cause a reduction in tremor similar to that observed in hyperthyroid mice.

Discussion

Previously it has been shown that peripherally-acting (quaternary) anticholinergic drugs abolish the peripheral muscarinic effects of tremorine and oxotremorine, without reducing the intensity of tremor or hypothermia. This finding led to the suggestion that hypothermia, like tremor, was centrally mediated (Spencer, 1965). The present finding, that peripheral muscarinic effects such as salivation and diarrhoea (which must be associated with marked heat losses) are not necessarily followed by the appearance of hypothermia, tends to confirm this suggestion.

In addition to there being distinct central and peripheral components to the actions of tremorine and oxotremorine, it is known that tremor and hypothermia are mediated separately. The tremor produced by oxotremorine is not a shiver brought about by the impending hypothermia; Blockus & Everett (1957) showed that if an animal's body temperature is prevented from falling, for example by conducting the experiment in an incubator, there is no reduction in the tremor. This we have confirmed. It follows that the reduction in tremor intensity observed in hyperthyroid mice is not due to the simultaneous reduction in the level of induced hypothermia. Similarly, it is not essential to produce tremor before there can be a hypothermia, since the administration of centrally-acting muscle-relaxant drugs, such as chlordiazepoxide, will abolish the tremor without affecting the hypothermia present (Spencer, 1965).

Yet it is likely that the simultaneous reductions in tremor and hypothermia observed in hyperthyroid mice are due to a similar mechanism. For example, the conversion of tremorine to oxotremorine may be hindered in some way in hyperthyroid mice, although this would not explain why the peripheral effects of tremorine are not reduced, nor why there is a reduction of tremor and hypothermia due to injected oxotremorine. Similarly, it is not likely that the biological inactivation of oxotremorine is enhanced in hyperthyroid mice, because the intensity and duration of its peripheral effects are no less than in controls. Another explanation could be that penetration of oxotremorine into nervous tissue is impaired in hyperthyroid animals; at present there is no evidence to support or refute this suggestion. A more likely explanation is that central adrenergic mechanisms are enhanced in hyperthyroid mice. For example, Prange & Lipton (1962) showed that the toxicity of imipramine is increased in hyperthyroid mice. In these laboratories, we have observed that the pharmacological effects of amphetamine are potentiated in hyperthyroid mice. Also, there is ample evidence that peripheral adrenergic mechanisms are potentiated in hyperthyroid animals (Hess & Shanfeld, 1965), and it is not unlikely potentiation also occurs centrally. Previous work (Spencer, 1965; 1966; Morpurgo, 1967) has shown that

centrally-acting adrenergic drugs, such as amphetamine and the imipramine-like drugs, antagonize the effects of tremorine and oxotremorine, whereas peripherally-acting adrenergic drugs are inactive or far less active. Whether or not the observed antagonism of the central effects of tremorine and oxotremorine in hyperthyroid mice is due to an enhancement of central adrenergic mechanisms remains to be proved.

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