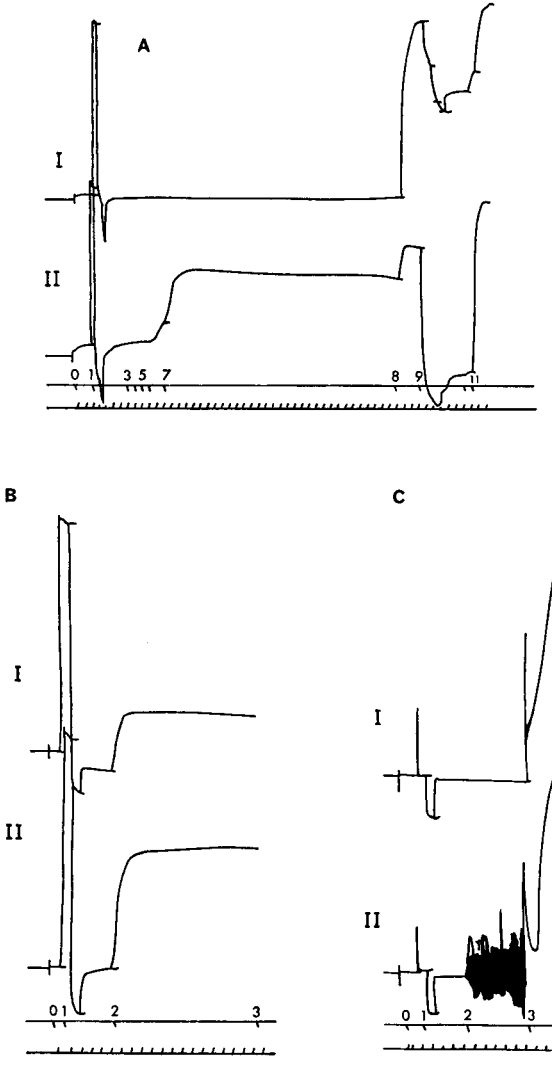
Calcium-dependent stimulating action of triiodothyronine on the isolated muscle of the frog

SIR,—The precise mechanism of action of the iodinated thyroid hormone or its substitutes is far from being clearly understood. Their generally delayed effects suggest some intricate, enzymatically induced actions. Very few immediate effects on isolated organs, tissues, or part of tissues, have so far been reported (Smith & Whalen, 1960; Lee, Lee & Yoo, 1965; Haldar, Freeman & Work, 1966).

We have found, however, that the rectus muscle of the frog, isolated in Ringer solution, is sensitive to relatively low concentrations of triiodothyronine, the threshold for inducing contracture usually being between 10 and 50 μM. In



addition, there are two procedures which enhance the effects of triiodothyronine on this muscle. These are (1) to keep the isolated recti in Ringer solution lacking calcium ions (Fig. 1A), or (2) to keep them in Ringer solution lacking potassium ions (Fig. 1B). Both procedures have in common the property of increasing the sensitivity of the muscles to triiodothyronine as well as to calcium chelating agents such as sodium edetate.

Under these sensitizing conditions, the threshold of the stimulating effect of triiodothyronine is lowered by at least five- to tenfold, and the amplitude of the induced contracture is increased two to four times.

In addition to the rectus, other skeletal frog muscles, like the pectoralis or sartorius, are sensitized by the same experimental procedures and actually the enhanced reactions follow individual response patterns, i.e. contracture, contracture with superimposed contractions, or bursts of repetitive contractions (Fig. 1C).

We may assume, theoretically at least, that ultimately the excitability of a given tissue is a direct or indirect function of the lability of its membrane-bound calcium ions. In our experiments, this concept is given support by the known chelating properties of the iodinated thyronine compounds (Lardy, 1955). It can be further tested by adding half of the stoichiometric amount of calcium ions, corresponding to the triiodothyronine concentration, to the solution of the muscle caused to contract in Ca-free medium. This addition reverses the contracture induced by the hormone, thereby suggesting that its action is calcium dependent.

If before addition to the Ringer solution, the triiodothyronine solution is treated with an adequate calcium chloride concentration and its effect on paired muscles compared with that of the untreated solution, we find that it has lost the greater part of its stimulating effect.

These muscle-stimulating actions of triiodothyronine are enhanced by cholinesterase inhibitors and by concentrations of barium chloride or caffeine which just fail to produce contraction. Conversely, sub-minimal concentrations of triiodothyronine potentiate the stimulation produced by caffeine or barium.

FIG. 1A. *Rana rid.* ♀, 110 g. Both recti muscles. Isotonic, vertically writing pens, amplifying 2 x under a pull of 2 g, in normal Ringer solution. Time marks = 30 min. 1. Acetylcholine 0·3 mM. After 15 min, at 2, muscle I was washed three times with Ringer solution, muscle II with Ca-free Ringer, both under a pull of 9 g lasting 30 min. At 3 to 7, both muscles received triiodothyronine 3 nM, 30 nM, 0·3, 3 and 10 μM. The first contracture occurred after 3 μM for the treated muscle in Ca-free solution, a concentration of triiodothyronine which produced no effect on the control. At 8, after 22 hr, a final reactivity test was made with 3 mM caffeine, followed by 3 washes as at 2. At 10, again 3 mM and at 11, 10 mM caffeine.

B. *Rana rid.* ♀, 88 g. Both recti muscles with the conditions described in A. 30 min after the initial acetylcholine test, the two muscles were washed 3 times: I with Ringer solution, II with K-free Ringer. At 2, both received a 30 μM concentration of triiodothyronine. At 3, 10 hr after beginning, the experiment was terminated.

C. *Rana rid.* ♀, 85 g. Both sartori muscles with the conditions as described in A. 30 min after the initial test to acetylcholine 0·3 mM, both muscles were washed 3 times, I with Ringer solution, II with K-free Ringer, both under a pull of 9 g lasting 30 min. At 2, both muscles received a 56 μM concentration of triiodothyronine. This produced a pronounced and rapid twitching in muscle II which persisted until the muscle was washed. 3 hr later, at 3, the final reactivity was tested with 5·6 mM caffeine.

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January 3, 1967

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References

Haldar, D., Freeman, K. & Work, T. S. (1966). *Nature, Lond.*, **211**, 9–12.
Lardy, H. A. (1955). *Brookhaven Symposia in Biology*, **7**, 90.
Lee, W. C., Lee, C. Y. & Yoo, C. S. (1965). *Br. J. Pharmac. Chemother.*, **25**, 651–657.
Smith, R. E. & Whalen, W. J. (1960). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **19**, 175.

Antagonism of morphine analgesia by reserpine and α-methyltyrosine and the role played by catecholamines in morphine analgesic action

SIR,—Since Vogt (1954) showed that morphine can deplete hypothalamic noradrenaline the participation of catecholamines in the analgesic action of morphine has been proposed by many authors (Radouco-Thomas, Radouco-Thomas & Le Breton, 1957; Schaumann, 1958; Paeile & Muñoz, 1966, and others).

The effect of reserpine on the analgesic action of morphine has now been examined by means of two different tests; also, the effect of pretreatment with α-methyltyrosine on morphine analgesia was investigated. According to current views (Spector, 1966) α-methyltyrosine is a potent inhibitor of tyrosine hydroxylase which leads to a selective depletion of brain noradrenaline and dopamine without affecting 5-hydroxytryptamine.

The increase induced by morphine (5 mg/kg, i.v.) of the pain threshold when the tooth pulp of the rabbit is electrically stimulated was abolished 24 hr after the administration of reserpine (2 mg/kg, i.v.). In the same way, chronic treatment with reserpine (0·2 mg/kg, s.c., once a day, during 14 days) much reduced the

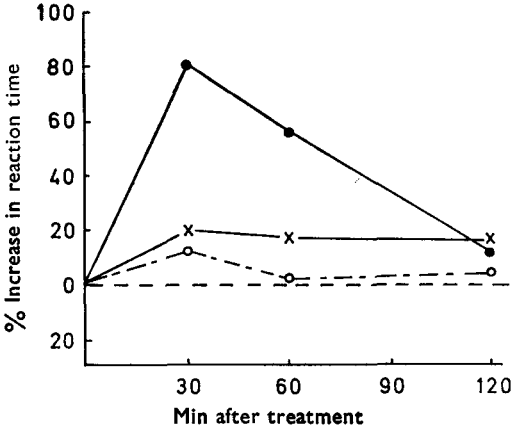

FIG. 1. Antagonism of morphine analgesic action by α-methyltyrosine in the mouse. Two doses of 100 mg/kg, i.p. of α-methyltyrosine were injected 8 and 4 hr respectively before morphine (10 mg/kg, i.p.). Analgesia was measured by the hot plate test (58° ± 0·5), according to Garcia Leme & Rocha e Silva (1961). The points on the curves represent the means of 40, 35 and 33 animals in the groups treated with morphine alone (●), morphine after α-methyltyrosine (×) or α-methyltyrosine alone (○), respectively.