Inotropic effects of thyroxine and related compounds on guinea-pig left atria *in vitro*

J. R. C. BAIRD AND B. A. SPILKER
Research Division, Pfizer Limited, Sandwich, Kent

Triiodothyronine, diiodothyronine and diiodotyrosine have positive inotropic activity on normal guinea-pig left atria *in vitro*. The increases produced by triiodothyronine and diiodothyronine are small but their detection shows that the inotropic responses to these agents can be studied *in vitro*. Thyroxine, thyronine and monoiodotyrosine are inactive. Reserpine pre-treatment reduces the inotropic effect of diiodotyrosine but does not reduce those of triiodothyronine and diiodothyronine.

Thyroxine has a positive inotropic effect in a variety of species including dog, cat and guinea-pig (Buccino, Spann, Pool, Sonnenblick & Braunwald, 1967; Piatnek-Leunissen & Olson, 1967; Goodkind, 1968; Braunwald, Sonnenblick, Spann & Buccino, 1969). These effects, however, have only been observed following *in vivo* administration, generally for several days, and there seem to be no reports of studies made *in vitro*. The *in vitro* effects of thyroxine (tetraiodothyronine), triiodothyronine, diiodothyronine, thyronine, diiodotyrosine and monoiodotyrosine on the inotropic responses of isolated left atria from normal and reserpinized guinea-pigs were determined. It was found that while thyroxine itself was inactive, some of the related compounds elicited a small positive inotropic response.

Methods.—Left atria from normal and reserpinized male guinea-pigs (400–700 g) were used. The animals were killed by a blow on the head, their hearts rapidly removed and the left atria mounted in well oxygenated muscle chambers containing Krebs-bicarbonate solution at 26° C. The preparation was stimulated at a frequency of 1 Hz, through a pair of platinum electrodes. Rectangular 1 ms pulses were used, and the voltage was 10–15% above threshold. The resting tension was 1 gramme. Contractions were recorded isometrically on a Devices recorder.

Some of the animals were injected with reserpine (5 mg/kg intraperitoneally) 18–24 h before the experiment.

Cumulative dose-response relations were determined with L-thyroxine (sodium salt, pentahydrate), 3,3',5-triiodo-L-thyronine (sodium salt), 3,5-diiodo-L-thyronine, L-thyronine, 3,5-diiodo-L-tyrosine and 3-iodo-L-tyrosine. Thyroxine, triiodothyronine, diiodothyronine and thyronine were dissolved with gentle heating and stirring in 0·1% w/v sodium carbonate solution. Changes in pH in the muscle chambers were always less than 0·7 pH units. Diiodotyrosine and monoiodotyrosine were dissolved in deionized distilled water with gentle heating and stirring. The concentration of each drug was expressed in molar terms.

All tests for significance of differences between means were carried out using Student's t test.

Results.—Neither thyroxine (1×10^{-5}M, $2{\cdot}7\times10^{-5}$M and 6×10^{-5}M; $n=7$) nor thyronine ($1{\cdot}4\times10^{-5}$M, $3{\cdot}7\times10^{-5}$M and $8{\cdot}3\times10^{-5}$M; $n=5$) increased inotropic responses above those due to equal volumes of sodium carbonate (0·1% w/v) alone. Triiodothyronine ($1{\cdot}4\times10^{-5}$–$8{\cdot}3\times10^{-5}$M; $n=9$) and diiodothyronine ($1{\cdot}4\times10^{-5}$–$8{\cdot}3\times10^{-5}$M; $n=6$) produced dose-related increases which were significantly ($P<0{\cdot}05$) greater than those produced by sodium carbonate (0·1% w/v) alone. The drug-induced increases minus control responses are shown in Fig. 1a.

Diiodotyrosine ($1{\cdot}4\times10^{-5}$–2×10^{-4}M; $n=6$) also increased the inotropic response of normal guinea-pig left atria and was the most active compound studied (Fig. 1a). Monoiodotyrosine had relatively little effect, producing an increase of 4·7% at $8{\cdot}3\times10^{-5}$.

Reserpinized guinea-pig left atria were used to investigate whether the positive inotropic effects of triiodothyronine, diiodothyronine and diiodotyrosine on normal guinea-pig left atria were due to the release of noradrenaline. The increases produced by triiodothyronine and diiodothyronine in reserpinized atria were not significantly lower than those produced in normal atria (Fig. 1b). In fact, the increase to the lowest dose of diiodothyronine ($1{\cdot}4\times10^{-5}$M) in reserpinized atria was significantly ($0{\cdot}02>P>0{\cdot}01$) greater than that produced in normal atria. Apart from the lowest dose, diiodotyrosine had significantly ($0{\cdot}01>P>0{\cdot}001$) less effect on reserpinized atria than on normal atria (Fig. 1b).

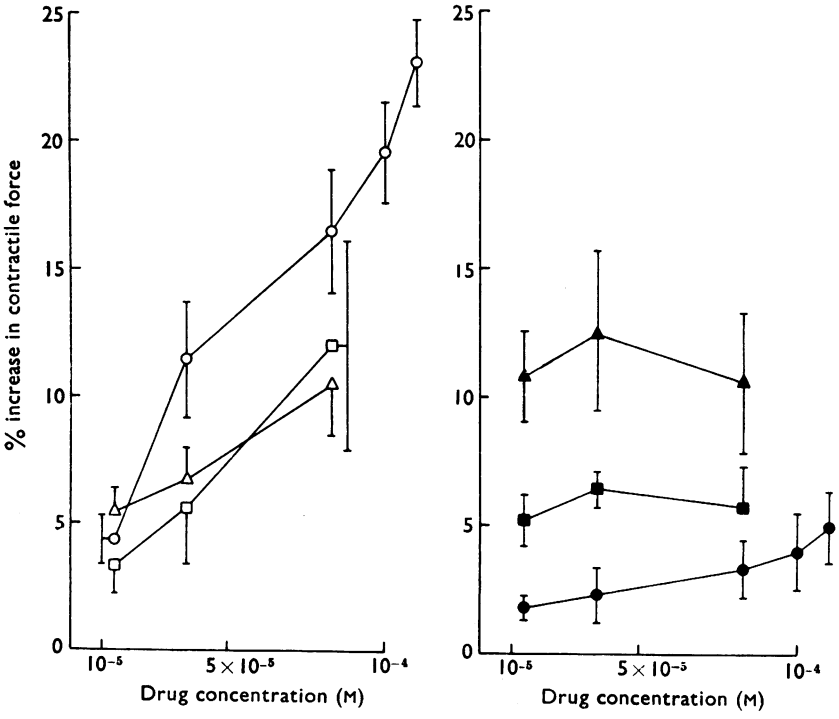

FIG. 1. Inotropic dose-response curves to diiodothyronine, triiodothyronine and diiodotyrosine in normal and reserpinized guinea-pig left atrial preparations. △, Diiodothyronine ± S.E.; □, triiodothyronine ± S.E.; ○, diiodotyrosine ± S.E.; ▲, diiodothyronine in reserpinized preparations ± S.E.; ■, triiodothyronine in reserpinized preparations ± S.E.; ●, diiodotyrosine in reserpinized preparations ± S.E.

Discussion.—The results demonstrate that both triiodothyronine and diiodothyronine have positive inotropic activity on normal guinea-pig left atria *in vitro*. The increases are relatively small but are statistically significant. The detection of the positive inotropic activity shows that the inotropic responses to these agents can be studied *in vitro*.

Diiodotyrosine probably produces its positive inotropic effect mainly by releasing noradrenaline since the responses were diminished in reserpine pretreated preparations. The positive inotropic actions of triiodothyronine and diiodothyronine, however, are not due mainly to the release of noradrenaline.

The positive inotropic activity *in vitro* of diiodothyronine and triiodothyronine and the inactivity of thyroxine, do not correlate with their thyromimetic activities.

The authors gratefully acknowledge the technical assistance of Mr. T. Kidd.

REFERENCES

BRAUNWALD, E., SONNENBLICK, E. H., SPANN, J. R. JR. & BUCCINO, R. A. (1969). Effects of heart failure, ventricular hypertrophy, and alterations in the thyroid state on the contractility of isolated cardiac muscle. *Ann. N.Y. Acad. Sci.*, **156**, 379–386.

BUCCINO, R. A., SPANN, J. R. JR., POOL, P. E., SONNENBLICK, E. H. & BRAUNWALD, E. (1967). Influence of the thyroid state on the intrinsic contractile properties and energy stores of the myocardium. *J. clin. Invest.*, **46**, 1669–1682.

GOODKIND, M. J. (1968). Left ventricular myocardial contractile response to aortic constriction in the hyperthyroid guinea-pig. *Circulation Res.*, **22**, 605–614.

PIATNEK-LEUNISSEN, D. & OLSON, R. E. (1967). Cardiac failure in the dog as a consequence of exogenous hyperthyroidism. *Circulation Res.*, **20**, 242–252.

(*Received August* 19, 1970)