Effects of dantrolene sodium on isolated skeletal, smooth and cardiac muscle of the guinea-pig

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Dantrolene sodium is a skeletal muscle relaxant that acts directly on the muscle fibres to impair the availability of calcium ions to the contractile mechanism. For a review of its actions and uses see Pinder, Brogden & others (1977). The drug is more effective in depressing skeletal than smooth or cardiac muscle (Ellis, Castellion & others, 1973; Butterfield & Ellis, 1973; Ellis, Simpson & others, 1975). Nevertheless, Meyler, Wesseling & Agoston (1976) reported that high concentrations of dantrolene depressed, by up to 75%, the amplitude of the beats of the isolated perfused rat heart that had been augmented by adrenaline. It was not clear from the results of Meyler & others (1976) whether dantrolene had depressed the basic contractility of the heart, or had antagonized the positive inotropic action of adrenaline in a more specific way.

The effects of dantrolene sodium were compared on isolated fully-curarized (tubocurarine 15 μg ml^{-1}) soleus muscles stimulated at a frequency of 0·1 Hz (Tashiro, 1973), on isolated spontaneously beating paired atria, on isolated left atria driven by electrical stimulation at a constant frequency of 2 Hz, and on isolated segments of tracheal smooth muscle, in which tone had been allowed to develop spontaneously with the passage of time (about 2 h after setting up the tissue). All preparations were obtained from guinea-pigs and were suspended in Krebs' solution (g litre^{-1}: NaCl, 6·95; KCl, 0·4; $CaCl_2$, 0·28; $MgSO_4.7H_2O$, 0·14; NaH_2PO_4, 0·14; $NaHCO_3$, 2·1; dextrose, 2·0) at 37° and gassed with a mixture of 5% CO_2 in oxygen. Isometric tension of all tissues was recorded with Grass force transducers (model FT03) coupled to a Grass (model 79) pen-recorder. Stock solutions of dantrolene sodium (3·99 mg ml^{-1} $\equiv 10^{-2}$M) were prepared in polyethylene glycol 400 and appropriate quantities were added to the organ bath from a microsyringe. In all experiments, control responses to equivalent concentrations of polyethylene glycol 400 alone were recorded.

Typical results obtained on all three tissues are shown in Fig. 1, and Fig. 2 graphically expresses the combined results from all experiments. The tension responses of all three tissues were depressed by dantrolene. The smallest effective concentration necessary to depress twitches of the isolated guinea-pig soleus muscle (about 10^{-6}M) was about 65 times greater than that necessary to depress twitches of the isolated rat diaphragm muscle ($1·5 \times 10^{-8}$M) recorded under the same conditions (Bowman, Khan & Savage, 1977). The log concentration-response curves for all tissues began to flatten, that

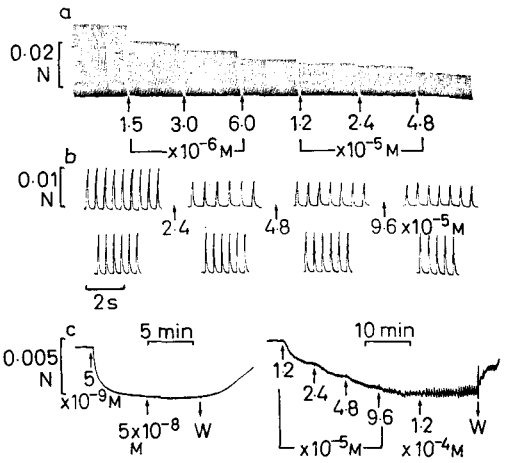

FIG. 1. Effects of dantrolene on (a) maximal twitches of the soleus muscle evoked at a frequency of 0·1 Hz, (b) spontaneous beats of paired atria, and (c) spontaneous tone of tracheal smooth muscle. In (a) and (b) the portions of the records shown illustrate the maximum depressions produced by the particular concentration of dantrolene. The numbers below the arrows denote the total cumulative concentrations of dantrolene, or, in the first panel of (c), of (−)-isoprenaline that was produced in the organ bath by successive drug additions. The tissues were not washed (except at W), so that the concentrations in the bath cumulated. The lower panels of (b) are from a different experiment and illustrate the absence of effect of equivalent concentrations of propylene glycol 400 on amplitude and frequency of beating. In (c), the oscillations in the relaxed tension of the tracheal muscle produced by high concentrations of dantrolene, were a regular finding; they did not occur with (−)-isoprenaline.

is the maximal responses obtainable were produced, with concentrations of dantrolene sodium about equivalent to that corresponding to its maximum solubility in water (i.e. 15 μg ml^{-1} or 3×10^{-5}M). Over 50% depression of the maximal twitches of the soleus muscle was produced by concentrations of dantrolene that were without effect on the atrial or tracheal muscle. However, larger concentrations of dantrolene produced steeper concentration-response curves in the tracheal and atrial muscles than in the soleus muscle (Fig. 2).

The results on guinea-pig atria are in qualitative agreement with those of Meyler & others (1976) who studied whole isolated hearts of rats. However, the maximum depression of the contractions of the spontaneously beating guinea-pig atria (about 55%) in our

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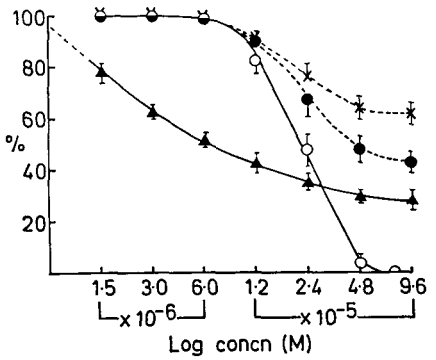

FIG. 2. Combined results illustrating the percentage depression, produced by dantrolene, of isometric twitch tension of the soleus muscle (—▲—, n = 9), isometric spontaneous beats of paired atria (- - -●- - -, n = 8), isometric driven beats of left atria (- -X- -, n = 8), and isometric spontaneously developed tone of tracheal smooth muscle (—○—, n = 6). Complete abolition of tracheal muscle tone, (i.e., 0%) was taken as being equal to the maximal relaxation produced by (−)-isoprenaline (see Fig. 1). The vertical bars are the standard errors of the means.

experiments was less than that of the contractions of the adrenaline-stimulated rat heart (about 75%) in their experiments. The difference was not due to the presence of adrenaline since, in a further series of experiments, we found that dantrolene produced the same percentage depression (maximum of about 55%) of paired guinea-pig atria in the presence of adrenaline (0·5 μg ml^{-1}), as it did in its absence. Left atria driven at a constant frequency (2 Hz), that approximately matched the spontaneous frequency, were depressed by dantrolene to a smaller extent (<40%) than were spontaneously beating paired atria. Dantrolene produced a small negative chronotropic effect in spontaneously beating atria (16%, s.e.m. 2·5, reduction in frequency of beating) which was maximal at all effective negative inotropic concentrations. This effect is evident in Fig. 1b and is probably sufficient to account for the extra negative inotropic effect in spontaneously beating atria, in accordance with the interval-strength (Treppe) relation (Koch-Weser & Blinks, 1963).

The most pronounced effect of high concentrations of dantrolene appeared to be produced on tracheal smooth muscle (Fig. 2) in which maximal relaxation matched that produced by (−)-isoprenaline (Fig. 1). However, it is perhaps not entirely justifiable to compare the responses of the three tissues in the way illustrated in Fig. 2, since the maximal degree of relaxation produced by (−)-isoprenaline is an arbitrary value not necessarily equatable with 100% relaxation. In another series of experiments in which tone of the tracheal muscle was produced by continuous contact with carbachol (0·06 μg ml^{-1}), the results indicated that, whereas isoprenaline in adequate concentrations produced complete abolition of carbachol-induced tone, dantrolene in concentrations of 7·5 × 10^{-5}M was maximally capable of producing only 54% (s.e.m. 9) inhibition. The effect of dantrolene on isolated tracheal smooth muscle differed from that on isolated skeletal and atrial muscle in that it was rapidly removed by washing the tissue (Fig. 1). In contrast, more than 2 h of repeated washing was necessary to restore the soleus muscle or atrial muscle contractions to normal, and in some experiments control amplitude was never regained. The relative ease with which the smooth muscle effect was removed by washing may be indicative of a more superficial site of action, or of a totally different mechanism of action. There is no evidence that the mechanisms of action of dantrolene on atria and on skeletal muscle are similar, although the different efficacies of the drug in the two types of muscle may merely reflect the different extents to which they rely on intracellularly stored Ca^{2+}. It is possible that the weak negative chronotropic action of dantrolene is also related to impairment of Ca^{2+} movement, since there is evidence that the autorhythmicity of the SA node is partly dependent on an inward calcium current (Cranefield, 1975).

Concentrations of polyethylene glycol 400 up to and including the maximum used as a solvent (0·2 ml in a 50 ml organ bath) when used alone slightly increased the twitch tension of the soleus muscle and the tone of the tracheal smooth muscle by mean values of less than 3%. The same concentrations were without measurable effect on the amplitude and frequency of the beats of the atria (Fig. 1b).

The important question, as Meyler & others (1976) have emphasized, is whether the cardiac depressant effects of dantrolene, demonstrable *in vitro*, might be important in patients. Ellis & others (1975) found dantrolene to be without effect on cardiac parameters in dogs, and, in unpublished experiments, Khan, Nott & Parratt have confirmed these negative observations in cats. However, it is possible that reflex sympathetic activity is an important factor in maintaining cardiac output in patients chronically treated with dantrolene; the results on isolated cardiac tissue show that adrenaline remains effective in the presence of the drug. The manufacturer's data sheet already warns that dantrolene should be used with caution in patients with severely impaired cardiac function due to cardiac disease. It is also possible that serious interaction on the heart might occur in patients concurrently treated with drugs that impair the activity of the sympathetic nervous system (e.g., adrenergic neuron blocking drugs and β-adrenoceptor blocking drugs), or that interfere with Ca^{2+} flux across cardiac cell membranes (e.g., verapamil or nifedipine). Experimental studies of such possible interactions would therefore be profitable.

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REFERENCES

BOWMAN, W. C., KHAN, H. H. & SAVAGE, A. O. (1977). *J. Pharm. Pharmac.*, **29**, 616–625.
BUTTERFIELD, J. L. & ELLIS, K. O. (1973). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **32**, 772.
CRANEFIELD, P. F. (1975). *The conduction of the cardiac impulse.* Mount Kisco, New York: Futura Publishing Co.
ELLIS, R. H., SIMPSON, P., TATHAM, P., LEIGHTON, M. & WILLIAMS, J. (1975). *Anaesthesia*, **30**, 318–322.
ELLIS, K. O., CASTELLION, A. W., HONKOMP, L. J., WESSELS, F. L., CARPENTER, J. F. & HALLIDAY, R. P. (1973). *J. pharm. Sci.*, **62**, 948–951.
KOCH-WESER, J. & BLINKS, J. R. (1963). *Pharmac. Rev.*, **16**, 601–652.
MEYLER, M. J., WESSELING, H. & AGOSTON, S. (1976). *Eur. J. Pharmac.*, **39**, 127–131.
PINDER, R. M., BROGDEN, R. N., SPEIGHT, T. M. & AVERY, G. S. (1977). *Drugs*, **13**, 3–23.
TASHIRO, N. (1973). *Br. J. Pharmac.*, **48**, 121–127.

Effect of tazolol on β-adrenoceptors in isolated preparations of the guinea-pig and rat

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The concept that β-adrenoceptors consist of two distinct sub-groups, designated β_1 and β_2 (Lands, Arnold & others, 1967) has been substantiated by reports in the literature describing agonists selective for β_2-adrenoceptors, e.g. salbutamol (Cullum, Farmer & others, 1969), and antagonists selective for either β_1-adrenoceptors, e.g. practolol (Dunlop & Shanks, 1968) and atenolol (Barrett, Carter & others, 1973), or β_2-adrenoceptors, e.g. butoxamine (Levy, 1966) and H35/25 (α,4-dimethyl-*N*-isopropylphenylethanolamine) (Levy, 1967). However, few if any compounds have been described which are selective agonists at β_1-adrenoceptors and we were therefore interested in the reports of Strosberg & Roszkowski (1972) and Lockwood & Lum (1974) which suggested that tazolol (1-isopropylamino-3-[thiazol-2-yloxy]propan-2-ol hydrochloride, formerly designated ITP) might fit this role. These groups of workers investigated the cardiovascular and metabolic effects of tazolol in anaesthetized animals (dogs and cats respectively) and concluded that the compound fitted into the category of a selective β_1-adrenoceptor stimulant. Subsequently, Strosberg & Buckley (1974) suggested that the cardiotonic properties of tazolol might be of value for the treatment of heart failure.

From the work described *in vivo* with tazolol, there is some uncertainty as to whether the compound is in fact a pure agonist. In view of these findings, we decided to investigate its activity on β-adrenoceptors using *in vitro* preparations. We chose isolated atrial and tracheal preparations from guinea-pigs (either sex, body weight range 320–570 g) and compared the β-adrenoceptor agonist activity of tazolol with isoprenaline and salbutamol on each tissue. The β_1-adrenoceptor stimulant activity was determined from increases in rate of spontaneously beating whole atria and from increases in the force of contraction of left atria electrically driven at a constant rate. The β_2-adrenoceptor stimulant activity was assessed from the inhibition of electrically-induced constrictions of the trachea.

Atrial preparations were suspended in McEwen's solution (1956) at 32°, aerated with 5% CO_2 in oxygen. A resting tension of 1 g was applied to each preparation and contractions recorded using an isometric force transducer (Dynamometer UF1). The contractions of spontaneously beating atria were used to trigger an instantaneous ratemeter. Stimulation of the isolated left atrium preparation consisted of square wave monophasic pulses of 0·5 ms duration and twice threshold voltage at a frequency of 2·25 Hz. The trachea was set up essentially as described by Farmer & Coleman (1970) to allow measurements of changes of intraluminal pressure in response to transmural electrical stimulation. This stimulation consisted of square wave monophasic pulses of 2 ms duration and supramaximal voltage applied to the tissue for 7 s periods at a frequency of 30 Hz. An interval of 2 min was allowed between each stimulation.

On each preparation, cumulative dose-response curves for the three drugs under investigation were obtained. The bathing fluid was changed between each drug and the order of addition of drugs was randomized between experiments. Since tazolol was suspected of having some antagonist activity, care was taken to make sure that the tissue washing procedure removed it from the bath so that the responses to any subsequent drugs administered were not impaired. This was achieved by administering an effective dose of isoprenaline before and afte tazolol. The responses to the drugs administered on each preparation were expressed as a percentage of the maximum response to isoprenaline and the results obtained are shown graphically in Fig. 1.

The maximum positive inotropic and chronotropic responses to tazolol on the guinea-pig atrial prepara-

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