Dantrolene sodium: effects on the spontaneous tone of guinea-pig urinary bladder detrusor muscle

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Dantrolene sodium, a peripherally acting skeletal muscle relaxant, has been used clinically in the treatment of chronic spasticity (Pinder et al 1977; Conte Camerino et al 1978). It is thought to affect skeletal muscle contraction by inhibiting trigger calcium (Putney & Bianchi 1974) or by directly inhibiting Ca^{2+} release from sarcoplasmic reticulum (Morgan & Bryant 1977). The mechanisms of action proposed suggest that other muscle contractile systems utilizing the same Ca^{2+} denominator could also be affected by the drug. However when it was first introduced it was thought to inhibit skeletal muscle contraction and have no significant effect on smooth or cardiac muscle (Ellis et al 1976). Nevertheless, recently it has been demonstrated that dantrolene sodium decreases the contractility of isolated heart preparations (Bowman & Khan 1977) and irreversibly depresses the contraction of the guinea-pig vas deferens and ileum preparations (Graves et al 1978). We have investigated the effects of the drug on spontaneous tone of guinea-pig detrusor muscle, in vitro. The bladder detrusor muscle preparations obtained from male guinea-pigs were mounted in an organ bath maintained at 37 °C and were superfused (Graves et al 1978) with aerated salt solution (NaCl 8·0, KCl 0·4, $CaCl_2$ 0·3, $MgSO_4.7H_2O$ 0·2, $NaH_2PO_4.H_2O$ 0·2, Na_2CO_3 0·1, glucose 1, ascorbic acid 0·05 g $litre^{-1}$; pH 7·3). Isometric tension of the tissue was measured with a force displacement transducer. The isolated muscle was perfused for 30–60 min to allow it to reach a steady state; after that time the perfusion solution was changed to one containing dantrolene sodium alone or with verapamil at the concentrations stated in the figures.

Dantrolene sodium in concentrations of 3·7, 7·5 and 15 $\mu g\ ml^{-1}$ caused a dose-related increase of the detrusor muscle tone. The coefficients of the regression line were the following: $y = 0{\cdot}1042 + 0{\cdot}0277 \times$ with a correlation coefficient of 0·67. The correlation coefficient was significant ($P < 0{\cdot}01$); degrees of freedom = 19. The maximum effect developed within 3·5 min and it was not reversible after washing the preparation with normal salt solution for more than 1 h (Fig. 1). The increase of the detrusor muscle tone caused by dantrolene sodium was antagonized by verapamil in a dose-related manner (Fig. 1).

Our results, in accordance with those of other investigators, show that dantrolene sodium interferes with smooth muscle. Smooth muscles from different organs have a varying morphology and behave differently (Burnstock 1970). It is known that dantrolene sodium chelates several ions (Morgan & Bryant 1977). In the present investigation the increase in the detrusor muscle tone it produced may be indicative of a more superficial site of action of the drug; Hurwitz et al (1967) showed that procedures which diminish the extracellular pool of Ca^{2+} lead to an increase in smooth muscle tone.

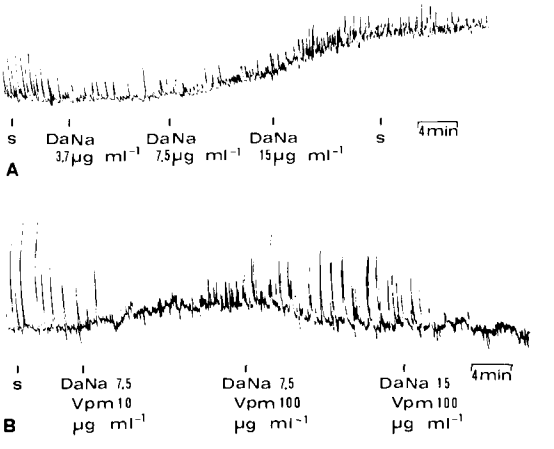

FIG. 1. Typical record of the effect of dantrolene (DaNa) on spontaneous tone of guinea-pig detrusor muscle, in vitro. In (A) at the arrows, the bathing medium (S) was changed to the stated concentrations of the drug. In (B) the effect of the drug is antagonized by verapamil (Vpm). At the arrows, the bathing medium was changed to one containing both drugs at the stated concentrations.

One of the side effects reported with the clinical use of dantrolene sodium is urinary incontinence (Pinder et al 1977; Graves et al 1978); this effect correlates with our results in vitro. Furthermore, our data show a correlation with early clinical observations of an investigation (still in progress in the Neurological Clinic of Bari) on patients with urinary retention of spinal origin (paraparesis due to spinal cord injury: 2 cases; paraparesis due to multiple sclerosis, 2 cases; tetraparesis due to spinal cord injury: 1 case). Dantrolene sodium (as Dantrium, Eaton Lab. Inc. Norwich, N.Y., USA) was given orally (300 mg day^{-1} on the average) to these patients, and remission of urinary retention was demonstrated by clinical and electrophysiological assessments, and by X-ray examinations of the urinary tract.

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An analysis of the inhibitory effects of quinine and mepacrine in the guinea-pig isolated ileum

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Quinine is an antimalarial agent which has in addition antipyretic and analgesic actions. Its toxic effects include tinnitus, vertigo, visual disturbances as well as gastrointestinal and respiratory disorders and even hypoprothrombinaemia.

Mepacrine is an antimalarial drug which is also used in the treatment of rheumatoid arthritis. Its anti-inflammatory properties might be related to its binding to various biological membranes (Lee 1971; Massari et al 1974) which, depending upon the membrane affected, promotes (i) lysosomal stabilization and inhibition of proteases extrusion in the inflamed tissue (Weissman 1968) (ii) inhibition of phospholipase A2 activity and reduction of the arachidonic acid concentration available for prostaglandin (PG) synthesis (Vargaftig & Dao Hai 1972; Flower & Blackwell 1976) (iii) inhibition of oxidative phosphorylation and mitochondrial ATPase activity (Hunter 1955; Whitehouse & Boström 1965). Both drugs are related in structure and properties to chloroquine, another antimalarial compound with antirheumatic properties (Famaey et al 1977a). All three antimalarials have been claimed to have in common a PG antagonist effect which might explain many of their properties (Manku & Horrobin 1976a, b; Horrobin et al 1977).

We have previously searched for this antagonistic effect of chloroquine in a guinea-pig isolated ileum preparation and we were unable to find any significant differences between the inhibitory effect of the drug on the contractile responses to several agonists including PG. We concluded that in that preparation chloroquine exhibits only a non-specific overall spasmolytic effect likely to be related to its membrane stabilizing properties (Famaey et al 1977a).

To check if this discrepancy between our results on the guinea-pig isolated ileum and those obtained in the rat vascular mesenteric bed by Horrobin et al (1977) was restricted to chloroquine, we have now extended our observations to quinine and mepacrine.

Contraction to PGE_1 (5 ng ml^{-1}, 45 s contact time, every 3 min), to histamine (30 ng ml^{-1}, 30 s contact time, every 6 min), to acetylcholine (20 ng ml^{-1}, 30 s contact time, every 3 min), to nicotine (0·5 μg ml^{-1}, 45 s contact time, every 6 min) and to 5-hydroxytryptamine (5-HT, 30 ng ml^{-1}, 45 s contact time, every 6 min) were elicited on guinea-pig isolated ileal segments of 4 cm length (removed at least 10 cm from the caecum) set up in Krebs Henseleit solution at 37 °C and gassed with a mixture of 5% CO_2 in oxygen. Similar ileal segments were set up in similar conditions and suspended under an initial load of 1 g. Isometric contractions (registered by a force transducer) were elicited by coaxial stimulation (pulse width 0·5 ms, pulse strength 5–25 V, frequency 0·1 Hz; Paton 1955).

At similar concentrations to those used previously with chloroquine (the lowest concentrations tested reducing both the electrically and the PG induced contractions by at least 50%; Famaey et al 1975, 1977a), quinine (5 μg ml^{-1}) or mepacrine (2·5 μg ml^{-1}) was added to the bath after 3 reproducible contractions of each agonist and the ileum was challenged again with the same agonists at the same intervals. When electrically induced contractions were used, the drug was added after 5 min of constant contractions. After 12 min contact time the antimalarial drug was washed from the bath and the ileum was again challenged at three consecutive intervals (during 6 min for electrical stimulations).

In another series of experiments, conducted simultaneously in a similar way, small amounts of PGE_2 or E_1 (2·5 ng ml^{-1}) were added to the bath 6 min after the antimalarial drug in an attempt to reverse the antimalarial inhibition. These were washed from the bath with the antimalarial drug 6 min later.

Finally, to determine the type of antagonism of the drugs dose response curves were constructed for acetylcholine, histamine and nicotine in the presence of quinine (5 μg ml^{-1}) or mepacrine (2·5 μg ml^{-1}).

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