Short communications

Influence of sodium pumping on smooth muscle contraction

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Isolated capsular muscle from cat spleen did not contract on exposure to a potassium-free bathing fluid. After exposure to conditions favouring downhill sodium and potassium movement (i.e. stimulation by noradrenaline in the absence of external potassium), subsequent reactivation of the sodium pump inhibited responses to noradrenaline. Under these special conditions, but not normally, the sodium pump may be behaving in an electrogenic manner.

The sodium pump in smooth muscle of rabbit urinary bladder and rat uterus has been shown to be electrogenic under special conditions which cause sodium enrichment of these muscles (Taylor, Paton & Daniel, 1970; Paton, 1971). These preparations were incubated first in cold potassium-free medium, with a consequent increase in tissue sodium. When the external potassium concentration was returned to the normal level sodium pumping was restored, resulting in hyperpolarization and inhibition of spontaneous mechanical activity. In smooth muscle cells under normal conditions the contribution of the electrogenic sodium pump towards the resting membrane potential is not clearly defined. If part of the resting membrane potential is due to activity of the pump then inhibition of the pump should result in depolarization. This possibility has been explored in the splenic capsular smooth muscle, which unlike the two preparations mentioned above, is not spontaneously active.

Methods.—Capsular strips (15 mm × 3 mm; 0·5 mm thickness) were cut with a Stadie-Rigg microtome from the spleens of cats weighing 1·5–2 kg. The strips were ordinarily bathed in Krebs-Henseleit solution (NaCl 118 mM, KCl 4·7 mM, $CaCl_2$ 2·5 mM, KH_2PO_4 1·1 mM, $MgSO_4$ 1·2 mM, NaHCO 25 mM, glucose 11 mM) kept at 37° C and equilibrated with 95% O_2: 5% CO_2.

Isometric tension changes were recorded with a Grass FT 03 C force-displacement transducer connected to a Grass Model 7 polygraph. Resting tension was adjusted to 1 g. For the potassium-free medium, potassium chloride was omitted from the Krebs-Henseleit solution and KH_2PO_4 was replaced with an equimolar amount of NaH_2PO_4. It was not thought necessary to correct for changes in osmolarity due to the small amount of KCl involved. Other substances used were (−)-noradrenaline bitartrate (Calbiochem) and ouabain (Nutritional Biochemical Co.).

Results.—Spleen strips from 10 cats showed no increase in tension when exposed to the potassium-free medium. The response to noradrenaline (1 μg/ml) did not appreciably change during the first 30–45 min of this treatment. Upon re-exposure to Krebs-Henseleit solution the resting tension did not change but the response to noradrenaline markedly decreased (Fig. 1A). This inhibition disappeared promptly on removal of external potassium (Fig. 1A) or after 30–45 min of continued exposure to normal external potassium concentration (Fig. 1B). The inhibition of the response to noradrenaline on re-exposure to potassium did not occur if the preparation was not stimulated with noradrenaline during the absence of potassium (Fig. 1C). A similar result was also obtained with histamine as the agonist. Pretreatment of the strips with ouabain (1 μg/ml), an inhibitor of sodium pumping (Skou, 1965), prevented the inhibitory effect of potassium on responses to noradrenaline (Fig. 1D).

Discussion.—In two smooth muscle preparations with an electrogenic sodium pump, viz. rat uterus and rabbit urinary bladder, removal of external potassium resulted in depolarization (Taylor *et al.*, 1970) and contraction (Paton, 1971). Absence of contraction in the spleen after similar treatment suggests that appreciable depolarization probably did not occur. The contribution, if any, of an electrogenic sodium pump to the resting membrane potential in this smooth muscle must therefore be small. However, repeated stimulation of the strips with noradrenaline in potassium-free medium followed by re-exposure to the normal medium containing potassium resulted in inhibition of responses to noradrenaline. The ability of

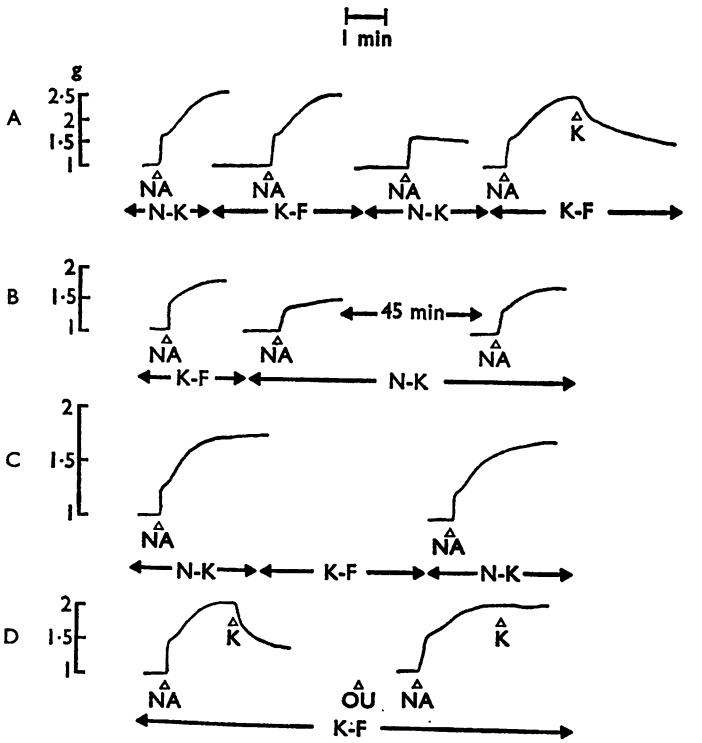

FIG. 1. Responses of strips of cat spleen capsule to noradrenaline (NA) (1 μg/ml) in Krebs-Henseleit solution (N-K) and potassium-free solution (K-F). Potassium chloride, 2 mM, was added at K, and ouabain, 1 μg/ml, at OU.

ouabain to prevent this inhibition suggests that the sodium pump may have a role in this phenomenon. An essential prerequisite for this inhibition seems to be prior stimulation of the muscle. This would be expected to increase smooth muscle membrane permeability and cause downhill movement of sodium and potassium ions. The absence of external potassium, by inhibiting the sodium pump, would be expected to favour this ionic imbalance. It is suggested that resumption of sodium pump activity under these conditions results in hyperpolarization which may account for inhibition of responses to noradrenaline. The removal of inhibition after prolonged exposure to potassium is probably due to restoration of normal sodium and potassium gradients. If this is true, it appears that an elevated intracellular sodium or decreased potassium activity is responsible for making the sodium pump electrogenic.

The method we have used to show an inhibitory role of the sodium pump, with stimulation of the muscle in the absence of potassium, is quicker and perhaps less drastic than the method of cold storage in a potassium-free medium used by Taylor *et al.* (1970) and Paton (1971).

From the above data it seems that the sodium pump in splenic capsular smooth muscle may be electrogenic under conditions favouring downhill sodium and potassium movements, but is not likely to be so under more normal conditions.

Supported by the Medical Research Council of Canada and the Manitoba Heart Foundation. Technical assistance of R. Weselake is gratefully acknowledged.

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(*Received February* 8, 1972)