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The effects of indomethacin on calcium, sodium, potassium and magnesium fluxes in various tissues of the guinea-pig

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Summary

1. Isolated tissues of the guinea-pig were bathed with Krebs solution at 37° C and subjected to 100 ms pulses of electrical stimulation for 30 min at a frequency of 0·1 or 1·0 Hz. The tissues were then dried, ashed, and the ash analysed for calcium, sodium, potassium and magnesium.

2. Gastric smooth muscle, cardiac and skeletal muscles and brain all showed a gain of sodium and calcium and a loss of potassium in response to electrical stimulation, but there was no significant change in the magnesium content of any of these tissues.

3. Indomethacin (0·5 mM) reduced the calcium content of unstimulated gastric smooth muscle and reduced the gain of calcium and sodium in response to electrical stimulation, but slightly increased the net loss of potassium in response to electrical stimulation.

4. Gastric smooth muscle which had gained calcium as a result of electrical stimulation, gradually lost it again when stimulation ceased. Indomethacin (0·5 mM) hastened the net loss of calcium from previously stimulated muscle.

5. Indomethacin (0·5 mM) failed to alter the calcium, sodium, potassium and magnesium contents of unstimulated cardiac muscle, skeletal muscle and brain. In these tissues indomethacin (0·5 mM) also failed to prevent the changes in the content of these minerals which occurred in response to electrical stimulation.

Introduction

Injured cells gain sodium and calcium and lose potassium. These chemical changes are the consequence of the primary injury, but they may, in their turn, inflict further damage on the cell. The accumulation of calcium, in particular, produces detrimental effects on several cellular functions (Slater & Cleland, 1953; Gallagher, Gupta, Judah & Rees, 1956; Calvert & Brody, 1958; Lehninger, 1962; Reynolds, Thiers & Vallee, 1962; Epstein & Whittam, 1966; Lehninger, 1970; Van Rossum, 1970; Wrogemann, Blanchaer & Jacobson, 1970; Romero & Whittam, 1971). Qualitatively similar changes in mineral composition, although usually reversible and smaller, follow excitation of most muscular and nervous tissues. Moreover, the accumulation of small amounts of ionic calcium within the cytoplasm of muscle cells is required to trigger the shortening of the contractile proteins (Somlyo & Somlyo, 1968, 1970). It may be significant, therefore, that indomethacin and a group of pharmacologically related drugs which are used

clinically to treat various forms of tissue injury also inhibit contraction of smooth muscle in response to a variety of spasmogenic agents (Northover, 1967a, b, 1968) and this inhibitory action can be attributed to the prevention of the net gain of calcium by the muscle cells (Northover, 1971). The experiments to be reported here were designed to investigate whether, in electrically stimulated smooth muscle, indomethacin prevents the net fluxes of other cations besides calcium, and were extended to include similar observations on certain other tissues.

Methods

Albino guinea-pigs weighing 600–800 g were killed by a blow on the head. The required tissues were removed and rinsed with Krebs solution. Four longitudinal strips of mucosa-free muscle were cut from each stomach and some of these were used without further subdivision, as described previously (Northover, 1971). In other experiments the strips of gastric muscle were minced with scissors into pieces approximately 1 mm in diameter. Cardiac muscle (ventricular), brain (cerebrum) and skeletal muscle (gluteus maximus) were cut into slices 0·5 mm wide. The slices were then minced with a scalpel so that the final product consisted of pieces approximately 0·5 mm in diameter.

The minced tissues were incubated for 2 h at 37° C in approximately 1,000 times their volume of Krebs solution having the following composition (mM): NaCl 118; KCl 4·7; KH_2PO_4 1·2; $MgSO_4$ 1·2; $NaHCO_3$ 2·5; glucose 11; $CaCl_2$ 0·4 (unless otherwise stated) and gassed with 95% oxygen and 5% carbon dioxide. Samples of tissue to be stimulated electrically were transferred by pipette in 20 ml of bathing fluid to a rectangular Perspex trough (internal dimensions 1×5 cm) maintained at 37° C and kept stirred by a stream of bubbles of 95% oxygen and 5% carbon dioxide. Square wave pulses of current (16 mA, 100 ms, 0·1 or 1·0 Hz) from an electronic stimulator (Scientific & Research Instruments, model 6051) were delivered for 30 min via platinum electrodes placed at the two ends of the trough. Despite the high current density the temperature and pH of the bathing fluid showed no detectable change during stimulation.

At the end of the period of electrical stimulation, unless otherwise stated, the suspension of minced tissue was transferred to a centrifuge tube, the fragments of tissue allowed to sediment for 20 s and the bathing fluid decanted. In some experiments the tissues were incubated at 37° C in Krebs solution for a further period of up to 90 min after electrical stimulation had been discontinued to determine the reversibility of the changes in mineral composition produced by electrical stimulation.

The mineral composition of the tissues was determined after a brief rinse (30–50 s) with calcium-free Krebs solution. The tissues were lightly blotted, transferred to a tarred crucible for drying to constant weight (10–100 mg) at 100° C overnight, and then ashed in a muffle furnace at 600° C for 1 hour. The cooled ash was dissolved in a known volume (1–4 ml) of 1 N HCl solution containing 0·3 mM $LaCl_3$. The calcium and magnesium concentrations in this solution (or a suitable dilution of it) were determined by atomic absorption spectrophotometry (Unicam model SP 90A) at 423 and 285 nm respectively, whilst sodium and potassium concentrations were determined by flame emission spectrophotometry in the same instrument at 589 and 766 nm respectively. All mineral contents are expressed as μg atoms/g dry weight of tissue.

TABLE 1. *Changes in mineral content of gastric smooth muscle in response to electrical stimulation and to indomethacin*

Concentration of indomethacin (mM)	Frequency of stimulation (Hz)	Mineral content (μg atoms/g±S.E.) Unstimulated	Mineral content (μg atoms/g±S.E.) Stimulated	Mineral uptake (+) or loss (−) in response to stimulation (μg atoms/g)
		Calcium		
Intact strips				
0	0·1	2·02±0·13	3·34±0·20	+1·32*§
0·1	0·1	1·98±0·14	3·17±0·16	+1·19*
0·5	0·1	1·96±0·11	2·25±0·15	+0·29*§
Minced muscle				
0	0·1	3·87±0·16 π	5·07±0·21	+1·20*†
0	1·0		7·60±0·26	+3·73*‡
0·1	0·1	3·80±0·15	4·96±0·22	+1·16*
0·1	1·0		7·45±0·28	+3·65*
0·5	0·1	3·29±0·12 π	3·67±0·16	+0·38†
0·5	1·0		4·23±0·19	+0·94*‡
		Sodium		
Intact strips				
0	0·1	760±49	1,114±58	+354*§
0·1	0·1	757±32	988±45	+231*
0·5	0·1	763±35	856±30	+ 93§
Minced muscle				
0	0·1	776±30	993±46	+217*†
0	1·0		1,238±56	+462*‡
0·1	0·1	774±44	996±48	+222*
0·1	1·0		1,201±52	+427*
0·5	0·1	807±57	909±44	+102†
0·5	1·0		1,005±49	+198*‡
		Potassium		
Intact strips				
0	0·1	398±28	230±20	−168*
0·1	0·1	405±31	217±22	−188*
0·5	0·1	386±25	175±16	−211*
Minced muscle				
0	0·1	330±21	214±23	−116*
0	1·0		192±17	−138*
0·1	0·1	325±26	219±22	−106*
0·1	1·0		201±19	−124*
0·5	0·1	360±23	175±14	−185*
0·5	1·0		168±10	−192*
		Magnesium		
Intact strips				
0	0·1	39·3±2·2	42·1±2·5	+2·8
0·1	0·1	38·7±1·7	37·6±0·9	−1·1
0·5	0·1	39·8±3·0	38·5±1·2	−1·3
Minced muscle				
0	0·1	40·5±2·3	45·1±4·1	+4·6
0	1·0		34·8±1·8	−5·7
0·1	0·1	39·9±3·5	41·2±4·0	+1·3
0·1	1·0		42·9±2·6	+3·0
0·5	0·1	42·5±3·8	45·7±2·7	+3·2
0·5	1·0		40·6±3·2	−1·9

Uptake or loss values marked * are significantly greater than zero ($P<0·05$). A significant difference ($P<0·05$) exists between two uptake (or loss) values in the presence and absence of indomethacin for a particular element marked π §, † or ‡.

Results

Gastric smooth muscle

Calcium

The calcium content of unstimulated minced muscle was higher than that of unstimulated intact strips of muscle (Table 1), suggesting that the trauma of mincing caused an uptake of calcium. The calcium content of both intact and minced muscle was increased, however, by electrical stimulation at 0·1 or 1·0 Hz for 30 min, the higher frequency causing a greater net gain of calcium than the lower frequency (Table 1). Despite the damage caused by mincing, electrical stimulation caused similar net uptakes of calcium in intact and minced muscle. These experiments were conducted at a calcium concentration of 0·4 mM. The calcium content of unstimulated minced muscle exposed to a higher concentration (2·0 mM) of $CaCl_2$ was 7·43 μg atoms/g, compared with 3·87 μg atoms/g for muscle exposed to 0·4 nM $CaCl_2$. Nevertheless, the tissue exposed to the higher calcium concentration took up only 0·18 μg atoms/g in response to electrical stimulation at 0·1 Hz for 30 minutes. For this reason the lower concentration of calcium chloride in the bathing fluid was used routinely for these experiments.

Indomethacin (0·1–0·5 mM) reduced the net uptake of calcium in response to electrical stimulation of both minced and intact muscle (Table 1). Indomethacin

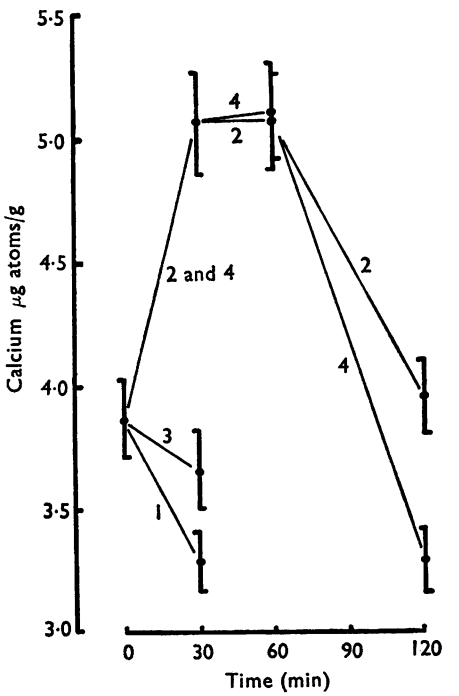

FIG. 1. Changes in calcium content of minced gastric smooth muscle in response to electrical stimulation (100 ms pulses, 0·1 Hz), with and without indomethacin (0·5 mM). 1. Treated with indomethacin for 30 min without stimulation. 2. Stimulated (0–30 min) in the absence of indomethacin and allowed to recover (30–120 min) in the absence of indomethacin. 3. Stimulated for 30 min in the presence of indomethacin. 4. Stimulated (0–30 min) in the absence of indomethacin and allowed to recover (30–120 min) in the presence of indomethacin. Vertical bars represent means ± standard error. The number of observations at each point was between 6 and 13.

had little effect on the calcium content of unstimulated intact muscle strips but reduced the calcium content of unstimulated minced muscle (Table 1).

Minced muscle which had accumulated calcium in response to electrical stimulation lost it again after a slight delay if stimulation was discontinued, provided that incubation in Krebs solution at 37° C was continued (Fig. 1). Addition of indomethacin to the bathing fluid after stimulation had ceased increased the rate of net loss of calcium so that 90 min after stimulation had ceased the minced muscle contained approximately the same amount of calcium as unstimulated indomethacin-treated muscle (Fig. 1).

Sodium and potassium

Electrical stimulation for 30 min at 0·1 or 1·0 Hz raised the sodium content and lowered the potassium content of minced gastric muscle (Table 1). The sodium content of unstimulated minced muscle was only slightly higher than that of unstimulated muscle strips whereas the potassium content of the former was noticeably lower than that of the latter (Table 1). These results suggest that the trauma of mincing was sufficient to increase the calcium and decrease the potassium contents but was insufficient to alter the sodium content appreciably.

TABLE 2. *Changes in mineral content of cardiac muscle in response to electrical stimulation and to indomethacin*

Concentration of indomethacin (mM)	Frequency of stimulation (Hz)	Mineral content (μg atoms/g±S.E.) Unstimulated	Mineral content (μg atoms/g±S.E.) Stimulated	Mineral uptake (+) or loss (−) in response to stimulation (μg atoms/g)
		Calcium		
0	0·1	8·73±0·15	8·80±0·18	+0·07
0	1·0		9·91±0·19	+1·18*
0·5	0·1	8·70±0·13	8·92±0·20	+0·22
0·5	1·0		9·93±0·17	+1·23*
		Sodium		
0	0·1	752±22	737±27	−15
0	1·0		864±20	+112*
0·5	0·1	775±25	782±22	+7
0·5	1·0		889±28	+114*
		Potassium		
0	0·1	212±18	169±12	−43
0	1·0		135±10	−77*
0·5	0·1	220±17	176±19	−44
0·5	1·0		128±16	−92*
		Magnesium		
0	0·1	30·4±1·8	33·8±2·8	+3·4
0	1·0		26·3±1·6	−4·1
0·5	0·1	31·3±2·3	28·8±3·0	−2·5
0·5	1·0		31·5±2·7	+0·2

Uptake or loss values marked * are significantly greater than zero ($P<0·05$), but in no case is the uptake or loss of an element in response to a particular frequency of electrical stimulation significantly greater or less in the presence of indomethacin than in its absence ($P>0·05$).

Addition of indomethacin (0·5 mM) to the bathing fluid caused no significant changes in the sodium and potassium contents of minced or intact muscle but reduced the net gain of sodium although not the net loss of potassium in response to electrical stimulation (Table 1).

Magnesium

Electrical stimulation for 30 min at 0·1 or 1·0 Hz failed to alter significantly the magnesium contents of minced or intact muscle and the addition of indomethacin (0·5 mM) to the bathing fluid likewise failed to produce a significant change (Table 1).

Sorbitol space

The sorbitol space has been shown by Goodford & Leach (1964) to be an indicator of the extracellular fluid space of smooth muscle. By their method, the sorbitol space of minced gastric smooth muscle was found to be 58 ± S.E. 6% of the wet weight of the tissue. After electrical stimulation (1·0 Hz, 30 min) the sorbitol space was not significantly changed either in the absence (54 ± S.E. 9%) or in the presence (54 ± S.E. 5%) of 0·5 mM indomethacin. The changes in mineral

TABLE 3. *Changes in mineral content of skeletal muscle in response to electrical stimulation and to indomethacin*

Concentration of indomethacin (mM)	Frequency of stimulation (Hz)	Mineral content (μg atoms/g ± S.E.)		Mineral uptake (+) or loss (−) in response to stimulation (μg atoms/g)
		Unstimulated	Stimulated	
		Calcium		
0	0·1	11·3 ± 0·22	11·6 ± 0·19	+0·3
0	1·0		12·7 ± 0·21	+1·4*
0·5	0·1	11·1 ± 0·15	11·0 ± 0·16	−0·1
0·5	1·0		12·6 ± 0·15	+1·5*
		Sodium		
0	0·1	881 ± 35	897 ± 41	+ 16
0	1·0		1,128 ± 53	+247*
0·5	0·1	892 ± 37	903 ± 29	+ 11
0·5	1·0		1,176 ± 47	+284*
		Potassium		
0	0·1	128 ± 11	108 ± 16	−20
0	1·0		90 ± 6	−38*
0·5	0·1	126 ± 9	108 ± 10	−16
0·5	1·0		92 ± 12	−34*
		Magnesium		
0	0·1	28·5 ± 1·7	26·2 ± 2·2	−2·3
0	1·0		27·8 ± 2·4	−0·7
0·5	0·1	27·1 ± 2·0	30·0 ± 2·3	+2·9
0·5	1·0		27·3 ± 1·8	+0·2

Uptake or loss values marked * are significantly greater than zero ($P<0·05$), but in no case is the uptake or loss of an element in response to a particular frequency of electrical stimulation significantly greater or less in the presence of indomethacin than in its absence ($P>0·05$).

content produced by electrical stimulation and by indomethacin, therefore, are unlikely to be due to changes in extracellular fluid space.

Cardiac and skeletal muscles

In both tissues electrical stimulation for 30 min at 1·0 Hz raised the contents of sodium and calcium, lowered the content of potassium but had no effect on the content of magnesium (Tables 2 & 3). There was a slight net loss of potassium from both tissues in response to stimulation at 0·1 Hz for 30 min, but at this frequency no change in sodium and calcium contents occurred (Tables 2 & 3). Indomethacin (0·5 mM) failed to produce a significant change in the content of any of these minerals in the unstimulated tissues and failed to prevent the changes in mineral composition which occurred in response to electrical stimulation (Tables 2 & 3).

Brain

Electrical stimulation for 30 min at 0·1 or 1·0 Hz raised the content of calcium and sodium, reduced the content of potassium, but left the content of magnesium unchanged (Table 4). The changes produced by the lower rate of stimulation were less than those produced by the higher frequency. The response of the brain to

TABLE 4. *Changes in mineral content of brain in response to electrical stimulation and to indomethacin*

Concentration of indomethacin (mM)	Frequency of stimulation (Hz)	Mineral content (μg atoms/g±S.E.) Unstimulated	Stimulated	Mineral uptake (+) or loss (−) in response to stimulation (μg atoms/g)
		Calcium		
0	0·1	3·44±0·19	5·16±0·27	+1·72*
0	1·0		6·38±0·28	+2·94*
0·5	0·1	3·20±0·16	5·51±0·30	+2·31*
0·5	1·0		6·29±0·29	+3·09*
		Sodium		
0	0·1	824±32	914±41	+ 90
0	1·0		1,016±46	+192*
0·5	0·1	881±36	964±48	+ 83
0·5	1·0		1,012±40	+131*
		Potassium		
0	0·1	178± 9	125±10	−53*
0	1·0		108± 4	−70*
0·5	0·1	175±8	134± 5	−41*
0·5	1·0		113± 6	−62*
		Magnesium		
0	0·1	35·3±3·0	35·8±2·6	+0·5
0	1·0		38·1±2·9	+2·8
0·5	0·1	36·2±2·5	32·5±2·3	+0·3
0·5	1·0		34·5±3·2	−1·7

Uptake or loss values marked * are significantly greater than zero ($P<0·05$), but in no case is the uptake or loss of an element in response to a particular frequency of electrical stimulation significantly greater or less in the presence of indomethacin than in its absence ($P>0·05$).

electrical stimulation resembled that of smooth muscle in that the net uptake of calcium was large even at the lower frequency of stimulation (Tables 1 & 4). In striated muscle, on the other hand, the net uptake of calcium was less than 20% of the original content of this element, even at the higher frequency of stimulation (Tables 2, 3 & 4). Brain differed from smooth muscle, however, in that electrical stimulation of the former produced a much smaller percentage change in sodium content than in calcium content (Tables 1 & 4).

Discussion

No previous studies of the action of indomethacin on the mineral composition of mammalian tissues appear to have been made but several investigators have studied the effects of other analgesic-antipyretic agents on the metabolism of metallic cations. Sodium salicylate depletes the rat isolated diaphragm of potassium (Keleman, 1960 ; Hicklin, 1963). Keleman (1960), in agreement with several earlier workers, reported that sodium salicylate also depleted erythrocytes of potassium, but Hicklin (1963) failed to confirm this finding. In those situations where potassium depletion has been observed with salicylate, an associated accumulation of sodium has also been reported. Aikawa & Reardon (1966) reported that sodium salicylate depleted several rabbit tissues of magnesium. Comparable 'downhill' net fluxes of cations were not produced by indomethacin in the present experiments. In contrast, it was possible to demonstrate, in smooth muscle at least, considerable protection against the 'downhill' net fluxes of sodium and calcium. It is perhaps noteworthy that in electrically stimulated smooth muscle the net loss of potassium was slightly increased by indomethacin treatment.

In several tissues, compounds with local anaesthetic activity inhibit to approximately the same extent the net gain of sodium and calcium, and also the net loss of potassium, in response to a variety of excitatory or damaging agents (Gallagher *et al.*, 1956 ; Rees, Sinha & Spector, 1961 ; Feinstein, 1963 ; Judah, Ahmed & McLean, 1964; Feinstein & Paimre, 1969; Chan & Quastel, 1970). The action of indomethacin on cation fluxes seems to differ from that of local anaesthetics in two respects. First, the only tissue studied in the present experiments which responded to indomethacin with a change in net mineral flux was smooth muscle. Secondly, the action of indomethacin on the net fluxes of metallic cations in smooth muscle showed some ion selectivity. The net uptake of sodium and calcium by electrically stimulated muscle was inhibited, whereas the loss of potassium was slightly increased. No explanation can be offered at the moment for either the ion or the tissue selectivity of the action of indomethacin.

The results of the experiments reported here are consistent with the suggestion that the protective actions of indomethacin in injured tissues are due, in part at least, to prevention of calcium accumulation in the injured tissue. The observations of Berczi & Somogyi (1969) and of Somogyi, Berczi & Selye (1969) are also relevant to this hypothesis. These workers noticed that certain inflammatory reactions of rat skin are associated with prominent calcification that is inhibited by sodium salicylate even more completely than are the other manifestations of the inflammatory responses. Experiments are now being done in this laboratory to extend the present observations to other cell types, including vascular endothelium, and to locate the subcellular site of calcium accumulation which is inhibited by anti-inflammatory drugs.

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