administration of the two indomethacin formulations and placebo are shown in Table 1. Statistical analysis of the values using Student's *t*-test showed that at 1 and 3 h after administration there was a highly significant fall ($P < 0.001$) in the CFF value in the subjects receiving the conventional capsule formulation compared with an equivalent dose of microencapsulated indomethacin. A significant lowering of the CFF was also observed at these times with the conventional capsule compared with the placebo. At 5 and 7 h the CFF values for the conventional capsule were still significantly lower than ($P < 0.05$) those of the microencapsulated drug or placebo. There was no significant difference between the values for the microencapsulated indomethacin and placebo at any time. The degree of central nervous activity as measured by critical flicker fusion frequency is thus more pronounced in subjects receiving the conventional indomethacin formulation than an equivalent dose of the drug in a microencapsulated form. These results would seem to support the unpublished observations of Dr M. Aylward of an increased incidence of c.n.s. side effects with conventional indomethacin capsules compared with equivalent doses of microencapsulated indomethacin. As the two formulations are bioequivalent, the mechanism of the reduced incidence of c.n.s. activity encountered with the microencapsulated product is unknown but illustrates the effect of dosage form on the incidence of c.n.s. side effects encountered with this particular drug.

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The influence of calcium utilization on cardiac cyclic AMP

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Rapp & Berridge (1977) proposed that calcium and cyclic (c)AMP interact in several tissues by the use of two feedback loops which they called loop A and loop B. In loop A, an increase in cAMP content results in an increase in intracellular calcium. On the other hand an increase in intracellular calcium results in a decrease in cAMP content. Such a loop would be ideal for a tissue where cAMP might accommodate the rate of entry of calcium into a cell for subsequent utilization, i.e., muscle contraction or secretion. In loop B, these interactions would be reversed. An increase in cAMP would result in sequestration or removal of intracellular calcium. An increase in intracellular calcium would trigger an increase in cAMP synthesis. Such a system would be ideal for situations where cAMP resulted in relaxation. Rapp & Berridge suggested, however, that both smooth and cardiac muscle utilized a feedback loop similar to loop B.

Meisheri et al (1978) studying isoprenaline-induced relaxation of the oestrogen-primed uterus noted that, while increased extracellular calcium markedly inhibited the ability of isoprenaline to increase uterine cAMP, there was no effect upon basal levels of cAMP in this tissue. The data would imply that no feedback loop exits for the modulation of basal cAMP levels by calcium in the oestrogen-primed uterus. In addition, the influence of calcium on cAMP in this tissue would appear to be opposite from that which would be expected if, as according to Rapp & Berridge, cAMP were involved in relaxation.

The purpose of the present study has been to determine if altered calcium utilization in cardiac tissue could result in altered basal and/or drug-induced levels of cAMP content.

Methods

Guinea-pigs of either sex, 300–500 g, were treated with heparin (3·23 mg kg^{-1} s.c.) 30 min before being stunned by a blow to the head and exsanguinated. Thoracotomy was performed, the hearts were excised and immediately placed in oxygenated Chenoweth-Koelle solution (CKS) of the following composition (mM) NaCl, 120; KCl, 5·63; $CaCl_2$, 2·0; dextrose, 9·7; $MgCl_2$, 2·0; $NaHCO_3$, 25·0.

After separation from the ventricles, left atria were cut into two strips and mounted vertically in tissue chambers containing 50 ml CKS which was continuously oxygenated with 95% O_2, 5%CO_2. One

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portion of each of the atria was secured vertically to an electrode made of acrylic plastic modified from that developed by Blinks (1966). The other portion was connected to a Grass FT-03 strain-gauge transducer.

The left atrial strips were quiescent unless excited electrically through two platinum electrodes situated such that the acrylic plastic clamp secured the lower portion of the atrial tissue agianst the heads of the electrodes. Spontaneously beating left atrial preparations were discarded. The muscles were stimulated at a frequency of 1·6 Hz with stimuli of 3ms duration at a voltage approximately twice threshold (1 to 3 V). Preliminary studies using propranolol demonstrated that this intensity range did not release significant quantities of endogenous catecholamine (Tenner & McNeill 1978).

The atria were allowed to equilibrate for 45 min under a mechanically applied resting tension of 1 g at 37 °C. During equilibration the bathing solution was replaced every 10 min. Isometrically developed force was recorded on a Grass model 79 polygraph. Developed force is reported as grams ± standard error of the mean.

After the equilibration period, the atrial strips either received 6 mM $CaCl_2$, D-600 (10^{-7} or 10^{-6} M) or isoprenaline (10^{-6} M). If the strip received 6 mM $CaCl_2$ or D-600, the tissue was allowed to equilibrate 15 min or until a steady state of developed force was achieved. At the end of this period, the atrial strips were rapidly frozen in 2-methyl butane cooled in dry ice which took between 5 and 8 s. When isoprenaline was to be tested, the strips were frozen at either zero, 15, 30 or 60 s after administration of the drug. The atrial strips were then wrapped in double layers of aluminium foil and stored in liquid nitrogen until assayed.

cAMP was extracted from the atrial strips according to Meisheri & McNeill (1977) and then measured by using a competitive protein binding kit purchased from Amersham/Searle. The values are reported as p mol mg^{-1} tissue wet weight.

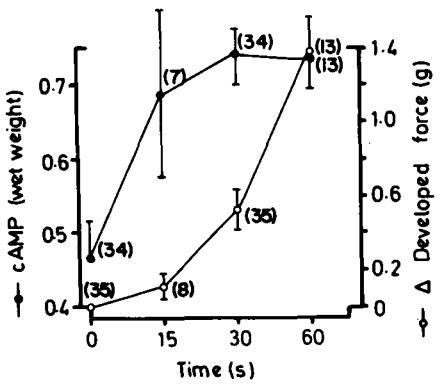

FIG. 1. Temporal relationship between changes in cardiac cAMP and the inotropic effect of isoprenaline in guinea-pig left atrial strips.

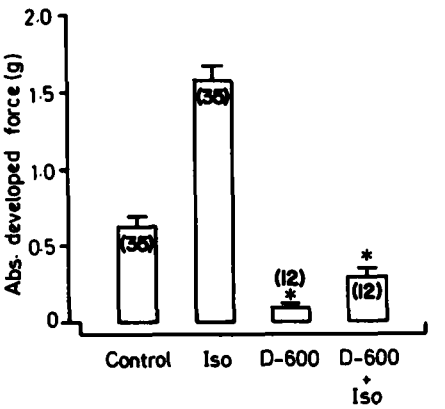

FIG. 2. The influence of D-600 on basal and isoprenaline-induced developed force. (*Significant ($P < 0.05$) depression of basal and drug-induced basal developed force). Both isoprenaline-treated groups produced inotropic effects significantly ($P < 0.05$) greater than their paired controls.

Statistical analysis was by Student's *t*-test for paired and unpaired data as indicated. A probability of $P > 0.05$ was chosen as the criterion for significance.

Results

When guinea-pig atrial strips were incubated for 15 min in the presence of either 6·0 mM calcium or the calcium channel antagonist, D-600, both basal developed force and cardiac cAMP levels were altered from control values (Table 1). In the presence of 6·0 mM calcium, both developed force and cAMP levels were increased by 58 and 59%, respectively while in the presence of D-600 (10^{-6} M) both were significantly depressed although to different extents (i.e. 86 and 26% respectively). In additional experiments (data not shown) D-600 (10^{-7} M) significantly decreased basal developed force (≃50%) but had no effect on basal cAMP levels.

The classical temporal relationship between cardiac cAMP content and developed force following the addition of isoprenaline (10^{-6} M) is shown in Fig. 1, the cAMP levels rising before the increase in developed force. The subsequent measurements in Figs 2 and 3 were obtained at 30 s, a time when cAMP changes were found to be maximal. Fig. 2 demonstrates that the calcium channel antagonist, D-600, significantly inhibited not only basal developed force but also the

Table 1. The influence of calcium on developed force and cardiac levels of cAMP.

	Developed force (g)	Cyclic AMP (p mol mg^{-1} wet wt)
Control (Basal values)	0·63 ± 0·03	0·462 ± 0·03
(n)	(35)	(34)
6·0 mM Calcium	1·08 ± 0·11a	0·776 ± 0·12a
(n)	(9)	(9)
D-600 (10^{-6} M)	0·09 ± 0·02b	0·343 ± 0·04b
(n)	(12)	(12)

a Significantly ($P < 0.05$) greater than control values.
b Significantly ($P < 0.05$) less than control values.

force developed in response to isoprenaline which itself increased developed force to a significant degree ($P > 0.005$) even in the presence of D-600. The increase in the presence of D-600 amounted to a doubling of the developed force. This was similar to the effect of isoprenaline in the absence of D-600.

Fig. 3 illustrates the depressant effect of D-600 (10^{-6} M) on both basal and isoprenaline-induced cardiac cAMP levels. The levels of cAMP in the D-600 treated tissues were significantly less than their respective controls (NCKS). In addition, D-600 abolished the stimulant effect of isoprenaline on cAMP levels. There is no significant difference between these in the presence of D-600 or D-600 + isoprenaline.

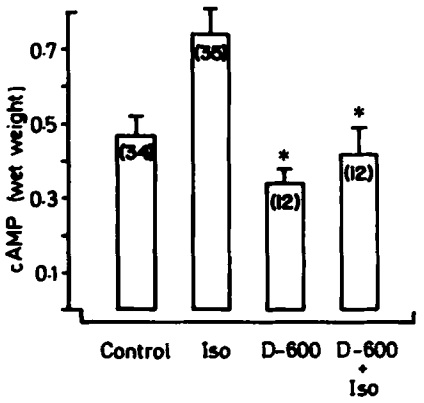

FIG. 3. The influence of D-600 on basal and isoprenaline-induced levels of cAMP. (*Significant ($P < 0.05$) depression of basal and drug-induced cAMP levels). There is no significant difference between the cAMP levels in the D-600 or D-600 + ISO treated atrial strips.

Discussion

The present study has demonstrated that basal levels of cAMP in guinea-pig left atrial strips could be either increased by elevating the extracellular calcium concentration or depressed by administration of a calcium channel blocker, D-600 (10^{-6} M). The development of force and cAMP levels were found to be directly proportional to calcium utilization. In addition, while D-600 antagonized the ability of isoprenaline (10^{-6} M) to increase developed force, it completely masked the ability of isoprenaline to significantly raise cAMP levels.

These data are not consistent with those found by Meisheri et al (1978) in the oestrogen-primed rat uterus. Indeed, their studies revealed no influence of calcium on basal cAMP levels and a reciprocal relationship between extracellular calcium concentration and the ability of isoprenaline to increase uterine cAMP levels (Meisheri & McNeill 1979). Comparison of the results of Meisheri et al (1978) with our findings would imply that two different modulation processes are at work in these two tissues. The concept proposed by Rapp & Berridge that smooth and cardiac muscle utilize the same feedback loop would appear not to be true when comparing oestrogen-primed uterus with cardiac tissue. The present results do, however, appear to confirm the presence of a loop B feed-back system in cardiac tissue.

There appears little agreement in the literature about the interaction between calcium and cAMP in cardiac tissue. Endoh et al (1976) reported that decreased frequency of stimulation, decreased extracellular calcium and D-600 (10^{-6} M) all resulted in an increase in the cAMP levels of rabbit papillary muscles. Harary et al (1976), studying rat cell cultures, also reported a reciprocal relationship between extracellular calcium and cAMP levels. The present studies, however, demonstrate, at least in guinea-pig left atria, that D-600 decreased, whilst elevated calcium increased, basal levels of cAMP. Hess & Gabel (1979), studying the influence of verapamil on perfused rat hearts, reported findings similar to ours. They noted that while verapamil decreased developed force in a dose-dependent fashion, the decrease in cAMP was not dose-dependent. They also reported that both effects were reversible in the presence of high calcium. Our data, as well as that of Hess & Gabel, would indicate that the ability of D-600 to depress basal levels of cAMP in guinea-pig left atrial strips is probably related to its ability to decrease calcium utilization by blocking calcium channels.

There is also disagreement in the literature concerning the effects of D-600 on drug-induced accumulation of cAMP. We found that D-600 depressed the contractile effect of isoprenaline proportionally to the decrease in basal developed force while also abolishing the ability of isoprenaline to increase cardiac cAMP levels. Harary et al (1976) reported that low calcium potentiated the ability of noradrenaline to increase cAMP levels. More recently, Johnson & Grupp (1979) reported that several calcium channel blockers inhibited both histamine- and isoprenaline-induced activation of adenylate cyclase in guinea-pig ventricles. Diltiazem and perhexiline also prevented specific [^{3}H]dihydroalprenolol binding to β-receptors. In contrast, the calcium antagonists did not alter basal or NaF-stimulated adenylate cyclase activity. It was concluded that the calcium antagonists act indirectly to perturb membrane lipids such that binding of receptors and agonists might be altered.

The possibility that the calcium antagonist, D-600, is having an effect other than as a pure calcium channel blocker cannot be ruled out in the present experiments. Indeed, while isoprenaline was able to produce a significant increase in developed force (a parameter that was more sensitive to the lower dose of D-600 (10^{-7} M) the increase in cAMP was completely inhibited. It could be speculated that the effect of D-600 on basal levels of cAMP is related to its ability to block calcium channels while its effect on drug-induced cAMP accumulation might be related to the non-specific effect described by Johnson & Grupp (1979).

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LETTER TO THE EDITOR

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The role of muscle and glial cells in potassium homeostasis

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There has not to date been any clearly assigned role for the β-adrenoceptors of the plasma membrane of skeletal muscles. It is known that adrenoceptors stimulate Na^+,K^+-ATPase in skeletal muscle (Cheng et al 1977). Recent experiments have shown that stimulation of the Na^+-K^+ transport system (Na^+-pumping) across muscle-cell membranes by a β-adrenoceptor-mediated mechanism contributes to the regulation of membrane potential and the distribution of Na-K across the sarcolemma (Clausen & Flatman 1977; Edstrom & Phillis 1981). β-adrenoceptor blockade can both potentiate the potassium rise in plasma during exercise and delay the reversion of plasma potassium to normal values (Carlsson et al. 1978) and we would like to suggest that muscle tissue plays a major role in the control of plasma potassium concentrations under normal physiological conditions. Catecholamines released by sympathetic nerves and the adrenal medulla would stimulate the muscle membrane sodium-potassium pump and thus accelerate the return of plasma potassium concentrations to normal following periods of muscular activity. Consistent with this suggestion is the observation that there is prolonged decrease in plasma potassium (hypokalaemia) after intravenous administration of adrenaline, which has been attributed to an increased uptake of potassium by muscle (Ellis 1956).

There is an adrenoceptor-mediated activation of Na^+-K^+ transport systems in glial cells of the central nervous system (c.n.s.) (Narumi et al 1979). It is known that glial cells are important for the maintenance of the homeostasis of extracellular electrolytes in the c.n.s. (Hertz 1977). By controlling extracellular potassium concentrations in the brain, glial cells would also influence the excitability of central neurons (Phillis 1979), since any decrease in extracellular potassium levels would lead to a subsequent hyperpolarization and thus inhibition of adjacent neurons. In this context it is interesting to note that adrenaline causes a decrease in extracellular levels of potassium in the brain (Pelligrino & Siesjo 1980) presumably as a result of enhanced sodium-potassium transport.

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