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Lack of inotropic selectivity of phosphodiesterase enzyme inhibitors in-vitro

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Phosphodiesterase enzyme (PDE) inhibitors form a diverse category of chemical structures which display positive inotropic activity, but it is unclear as to how force/rate selective these are on the heart. In this study several recently developed PDE inhibitors, almost all of which are selective for the cardiac PDE III isoenzyme, were compared with the xanthine PDE inhibitor IBMX and with ouabain and the direct and indirect adenylate cyclase activators, forskolin and isoprenaline, respectively. These compounds were active in increasing paced guinea-pig left atrial force, the order of potency being: isoprenaline > ouabain > forskolin > pimobendan > IBMX > sulmazole > buquineran > carbazeran > milrinone > piroximone > amrinone. Ro 13-6438 and enoximone were however, both inactive. With the exception of buquineran and carbazeran, which produced bradycardia and were therefore force specific, all of the recently discovered PDE inhibitors increased the rate of contraction of the right atria and they were rate selective to the same extent as IBMX, isoprenaline and forskolin. Sulmazole, the only force selective PDE inhibitor of the compounds studied, was intermediate between IBMX and ouabain in force/rate selectivity and pimobendan showed no selectivity. Since both these agents also possess other actions, then these results suggest that in general, PDE III inhibitors do not show advantageous force selectivity in guinea-pig atria.

The treatment of chronic heart failure has been dominated over the last century by cardiac glycosides in spite of their toxicity and controversial effectiveness (Hamer 1979; Johnston 1985). Catecholamines, such as dobutamine, dopamine and isoprenaline are powerful inotropic agents, but are of limited value due to the concomitant tachycardia, arrhythmogenicity and lack of oral efficacy (Goldberg et al 1977; Farah et al 1984). Recently, considerable interest has developed around a diverse class of chemical structures which demonstrate positive inotropic activity, a common mechanism of which appears to be inhibition of the cardiac phosphodiesterase enzyme (PDE) (Farah et al 1984). However, we are not aware of many in-vitro studies which have made a direct comparison of these agents with a catecholamine such as isoprenaline, to assess clearly their relative cardiac force/rate selectivities. The purpose of this study was to examine several new PDE inhibitors to assess their force/rate selectivities.

Materials and methods

Male guinea-pigs (300–400 g) were killed by cervical dislocation and the hearts rapidly removed. The left and right atria were mounted in 30 mL organ baths containing Krebs-Henseleit solution mM ($NaCl$ 117·6, $NaHCO_3$ 25, NaH_2PO_4 0·89, $MgSO_4$ 1·18, glucose 11·1, KCl 5·36, $CaCl_2$ 2·55) at 37 °C, gassed with 95% O_2, 5% CO_2. The atria were connected to Grass force displacement transducers (FT03) and maintained at a resting tension of 2 g. Right atria were allowed to beat spontaneously and the electrical recording of rate was triggered from the force signal. Left atria were electrically stimulated through platinum electrodes (1 Hz, 5 ms, 1·5 × threshold voltage). Right atrial rate and the force generated by both atria were recorded on a Devices M19 recorder.

After a period of equilibration, a conditioning and maximally effective concentration of isoprenaline (3×10^{-7} M) was given to each tissue to increase the rate and force of contraction. After the responses reached a maximum, the tissues were washed and allowed to stabilize. The administration of isoprenaline was repeated and the peak rate and force responses were taken as the maximum (i.e. 100%) response of the tissue. After washing and recovery, cumulative dose-response curves were obtained with one of the test compounds. The effect of each concentration was expressed as a percentage of the maximum response to isoprenaline. The various PDE inhibitors (listed below) were compared with an older PDE inhibitor, IBMX (3 isobutyl-1-methylxanthine) and with isoprenaline and forskolin (Bristow et al 1984), which, respectively, indirectly and directly stimulate adenylate cyclase, and with ouabain which stimulates the heart independently of cyclic (c) AMP involvement.

We gratefully acknowledge the gifts of forskolin (Hoechst), sulmazole and pimobendan (Thomae GmbH), milrinone and amrinone (Sterling Winthrop), buquineran and carbazeran (Pfizer), enoximone (MDL 17,043) and piroximone (MDL 19,205, Merrel-Dow) and Ro 13-6438 (*R*-6-chloro-1,5-dihydro-3-methyl-imidazo-[2,1-b]quinazolin-2[3H]-one, Hoffman La Roche). The following were purchased from commercial sources: isoprenaline hydrochloride (Pharmax Ltd), ouabain octahydrate (Sigma) and IBMX (Aldrich).

Stock solutions of the various drugs were obtained in the following ways. Isoprenaline was dissolved in saline with ascorbic acid as the antioxidant; IBMX, ouabain, buquineran and carbazeran in distilled water; sulmazole, piroximone, milrinone, amrinone and Ro 13-6438 in 0·1 M HCl; fenoximone in 0·3 M NaOH; pimobendan

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in polyethylene glycol (PEG 300) and forskolin in a mixture of PEG 300, ethanol and distilled water (1:1:1, v/v).

Results

The results obtained with the inotropically active compounds are shown in Fig. 1 and the responses are expressed relative to the isoprenaline-induced maximum response. Pimobendan, buquineran, carbazeran, milrinone and amrinone failed to increase the force of contraction by more than 50% of the isoprenaline-induced maximum, thereby differing from IBMX, sulmazole, piroximone and the adenylate cyclase activator forskolin, which either produced similar maximal stimulation to isoprenaline or showed no sign of a submaximal response. The order of the relative potencies and the pD_2 values and maxima are displayed in Table 1. Ro 13–6438 and enoximone failed to increase

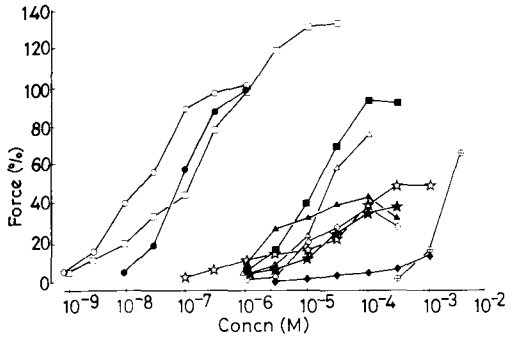

FIG. 1. Comparative positive inotropic activities of various compounds with isoprenaline in the paced left atrium (1 Hz, 37 °C) of the guinea-pig. Ouabain and forskolin are of a similar order of potency to isoprenaline whereas the PDE inhibitors are considerably less potent. Only the PDE enzyme inhibitors IBMX, sulmazole and piroximone are capable of producing a maximal inotropic stimulation similar to isoprenaline. The values shown are the mean of 4 experiments (the s.e.m. omitted for clarity) except for isoprenaline (n = 5) and sulmazole (n = 7). Key: isoprenaline ○, ouabain ●, forskolin □, IBMX ■, sulmazole △, pimobendan ▲, milrinone ☆, carbazeran ★, buquineran ◇, amrinone ◆, and piroximone ⊕.

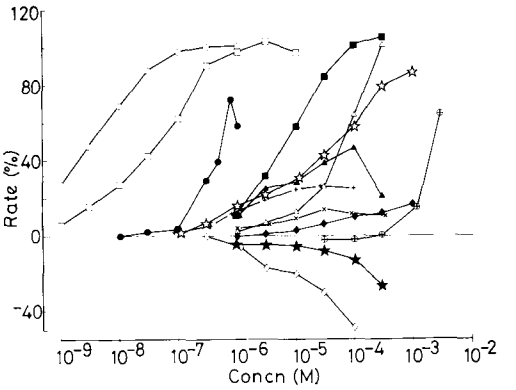

FIG. 2. Comparison of chronotropic activity in spontaneously beating guinea-pig right atria. All of the compounds, except buquineran and carbazeran increased atrial rate. Values are the mean of 4 experiments (the s.e.m. omitted for clarity) except for sulmazole (n = 8). In addition to those compounds shown in Fig. 1, enoximone × and Ro 13-6438 + are also included.

Table 1. Relative and absolute force and rate potencies in paced and spontaneously beating guinea-pig atria.

	Force			Rate	
Compound	Rel. Pot.	pD_2	Max (%)	pD_2	Max (%)
Isoprenaline	810	$7{\cdot}74 \pm 0{\cdot}21^b$	100	$8{\cdot}45 \pm 0{\cdot}08^b$	100
Ouabain	210	$7{\cdot}15 \pm 0{\cdot}04^b$	$>97 \pm 8$	$6{\cdot}31 \pm 0{\cdot}12^b$	$>80 \pm 5$
Forskolin	110	$6{\cdot}61 \pm 0{\cdot}24^b$	130 ± 8^a	$7{\cdot}45 \pm 0{\cdot}22^b$	102 ± 3
Pimobendan*	1·2	$4{\cdot}98 \pm 0{\cdot}41$	45 ± 6^b	$4{\cdot}98 \pm 0{\cdot}39$	47 ± 3^b
IBMX	1	$4{\cdot}83 \pm 0{\cdot}19$	96 ± 4	$5{\cdot}14 \pm 0{\cdot}04$	94 ± 6
IBMX*	1	$5{\cdot}20 \pm 0{\cdot}20$	—	$5{\cdot}63 \pm 0{\cdot}06$	—
Sulmazole	0·50	$4{\cdot}52 \pm 0{\cdot}17$	$>81 \pm 9$	$3{\cdot}97 \pm 0\ 13^b$	$>-102 \pm 15$
Buquineran*	<0·35	<5	37 ± 11^b	$4{\cdot}89 \pm 0{\cdot}34$	$<-47 \pm 9^b$
Carbazeran*	<0·29	$4{\cdot}66 \pm 0{\cdot}26$	38 ± 8^b	$<4^b$	$<-27 \pm 10^b$
Milrinone	0·035	$3{\cdot}37 \pm 0{\cdot}32^b$	$>50 \pm 4$	$4{\cdot}33 \pm 0{\cdot}11^b$	87 ± 3
Piroximone	0·013	$2{\cdot}94 \pm 0{\cdot}10^b$	$>65 \pm 12$	$3{\cdot}37 \pm 0{\cdot}15^b$	$>83 \pm 5$
Amrinone*	<0·006	$<3^b$	$>14 \pm 6$	$3{\cdot}12 \pm 0{\cdot}12^b$	$>23 \pm 4$
Ro 13-6438	NA	—	—	—	23 ± 5
Enoximone	NA	—	—	—	19 ± 6

Values are the mean ± s.e.m. of 4 experiments, except for isoprenaline (force, n = 5) and sulmazole (force, n = 7 and rate, n = 8). Compounds marked * are quoted at the 25% value (pD_{25}%), otherwise the pD_2 value (negative log of the EC50) is quoted. The relative potency for force is compared with IMBX using the mean values. NA signifies inactivity. [a] ($P < 0{\cdot}05$) and [b] ($P < 0{\cdot}01$) indicate the significance relative to the corresponding IBMX value using unpaired Student's *t*-test.

the inotropy of the paced atrium but this was not due to an opposing effect of the respective vehicles.

Most of the compounds also increased the spontaneous rate of beating of the right atrium as shown in Fig. 2 and Table 1. Only buquineran and carbazeran produced bradycardia despite their positive inotropic response. Ro 13-6438 and enoximone, which were both inotropically inactive did however cause a small degree of tachycardia. The force/rate selectivity of each of the compounds is shown in Fig. 3, in which the mean effect produced by each concentration of drug is expressed as a percentage of the isoprenaline-induced maximum for rate versus force. A hypothetical non-selective agent would therefore lie along the broken line. IBMX, isoprenaline and forskolin each lay to the right of this line, indicating that these may be considered rate

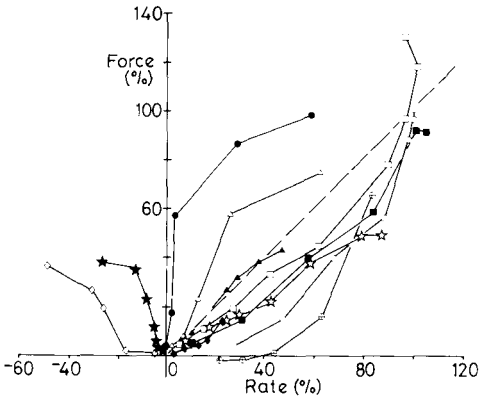

FIG. 3. Comparison of the force/rate selectivity of the PDE inhibitors using the same key as shown in Fig. 1. The responses are expressed as the mean percentage of the isoprenaline-induced maximum. A hypothetical non-force/rate selective compound would lie along the broken line and IBMX, isoprenaline, forskolin and most PDE inhibitors are near or to the right (rate selective) of this line. Sulmazole is the only force selective compound, whilst buquineran and carbazeran decrease rate at inotropically active concentrations and are therefore force specific.

selective agents on the basis of the distinction used in this study, in contrast to the cardiac glycoside, ouabain, which was highly force selective. Sulmazole was the only new generation PDE inhibitor to demonstrate force selectivity, though not to the same extent as ouabain. Pimobendan lay along the line thereby showing no selectivity. The other PDE inhibitors, milrinone and amrinone were of comparable selectivity to IBMX, and piroximone was the most rate selective of the inotropically active agents. The chemically related quinazolines, carbazeran and buquineran were the only PDE inhibitors to produce bradycardia at concentrations which increased inotropy.

Discussion

Cardiac glycosides, the traditional drugs used in the treatment of heart failure, are highly force selective, as confirmed by this study using ouabain. The lack of a pronounced direct effect on the sinoatrial node would give these inotropic agents an ideal pharmacological profile were it not for their poor therapeutic index, principally due to the production of arrhythmias, and their questionable value in patients with sinus rhythm (Hamer 1979; Johnston 1985).

Both older catecholamines and those more recently developed for the treatment of heart failure all suffer the common disadvantage of increasing heart rate, a direct consequence of the fact that both the inotropic and chronotropic mechanisms are common, relying on the stimulation of the adenylate cyclase system linked to the β-adrenoceptor. As a consequence tachycardia and arrhythmias are clinical disadvantages of these agents (Goldberg et al 1977; Farah et al 1984).

The more recently developed alternative positive inotropic agents designed in the hope of overcoming these disadvantages are non-catechol, non-glycoside compounds, many of which are reported to inhibit cardiac PDE (Farah et al 1984). Recent studies have identified these 'second generation' PDE inhibitors as selective for the cardiac PDE III isoenzyme (Weishaar et al 1985). On the basis of selective PDE III inhibition, it might be reasoned that it could be possible to introduce force/rate selectivity, thereby having compounds with more advantageous profiles than displayed by the older non-selective xanthine PDE inhibitors such as IBMX (Weishaar et al 1985) or of the adenylate cyclase activators isoprenaline and forskolin. From our results, this does not necessarily seem to be the case since, amrinone, milrinone and piroximone did not demonstrate any force/rate selectivity over IBMX, isoprenaline or forskolin.

Amrinone (Endoh et al 1982; Hayes et al 1984; Holck et al 1984), milrinone (Bristol et al 1984) and piroximone (Kariya et al 1984) are selective PDE III inhibitors and their in-vivo inotropic activity correlates well with this property (Bristol et al 1984). Both amrinone (Alousi & Dobreck 1983) and piroximone (Roebel et al 1984) have been compared with isoprenaline in-vitro in cat right atria and papillary muscle and a small degree of force/rate selectivity was demonstrated. However from this study in guinea-pig atria, there is little or no selectivity shown by amrinone, and piroximone is rate selective. Sulmazole was the only PDE inhibitor to demonstrate definite force/rate selectivity and this is in agreement with the findings of Dahmen & Greeff (1981) who compared sulmazole with another β-adrenoceptor agonist, orciprenaline, in guinea-pig atria. One possible important difference between sulmazole and the other compounds is that it is reported to be a selective inhibitor of the PDE II enzyme (Weishaar et al 1985) and in addition it increases the sensitivity of the myofilaments to calcium (Solaro & Ruegg 1982).

Pimobendan also offered better force/rate selectivity than the other PDE inhibitors, but to a lesser extent than sulmazole since it was non-selective. A large force/rate selectivity compared with isoprenaline was claimed for pimobendan by Berger et al (1985) using paced papillary muscle and spontaneously beating atria of the guinea-pig. Pimobendan is reported to elevate cAMP and has all the characteristics of a PDE inhibitor but in addition prolongs the action potential duration which may be an additional mechanism of its inotropic action (Honerjäger et al 1984).

The bradycardia produced by the quinazolines, buquineran and carbazeran, is an unusual (though attractive) feature of PDE inhibitors. Alabaster et al (1977) demonstrated that buquineran increased guinea-pig atrial force and reduced rate, thereby resembling their findings with ouabain. Buquineran was found to inhibit the PDE enzyme but since it did not affect Na^+,K^+-ATPase activity, then the mechanism of the bradycardia remains a mystery. In contrast to tachycardia caused only by high doses of buquineran in the dog, heart rate was not affected in man (Follath et al 1976). However QT prolongation, viewed as a potential source of arrhythmia, was noted in man (Russell et al 1979).

The lack of inotropic stimulation with both enoximone and Ro 13-6438 is strange in view of the reported activity in the paced guinea-pig left atrium (Roebel et al 1982; Eigenmann et al 1984) and no obvious explanation can be offered. The vehicles used with each drug had no activity of their own and therefore this cannot be the explanation for the lack of activity. Both compounds did however produce a small rise in atrial rate.

It may be dangerous to draw parallels from this in-vitro work to the in-vivo situation, particularly since PDE inhibitors are also vasodilators and therefore like isoprenaline, will induce baroreceptor reflex-mediated rate and inotropic changes. In addition any clinical benefit obtained in patients with heart failure may also stem from afterload reduction as well as from an inotropic effect. Milrinone and piroximone were reported to be inotropically selective when compared with isoprenaline in the chloralose-anaesthetized dog, but this selectivity was lost following ganglion block (Shaffer et al 1985). In a more recent study with isomazole, another PDE III inhibitor, no force/rate selectivity was demonstrated over isoprenaline in the pentobarbitone anaesthetized dog (Hayes et al 1986). Both of these in-vivo findings when performed in the absence of baroreceptor reflexes, concur with the lack of force/rate selectivity suggested by our atrial work. Clearly more in-vivo studies of this nature would be required to decide how relevant our in-vitro findings are.

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