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Inotropic and Chronotropic Effects of 4-(4′-*n*-Butylaniline)-7,8-dimethoxy-5H-pyrimido[5,4-*b*]indole in Guinea-pig Atria

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Abstract

Cardiotonic effect of 4-(4′-*n*-butylaniline)-7,8-dimethoxy-5H-pyrimido[5,4-*b*]indole (B_{11}) was investigated in isolated cardiac tissue preparations. The action of this agent on force of contraction, beating frequency and cyclic nucleotide phosphodiesterase (PDE) activity was studied. Amrinone was used for comparison.

B_{11} produced concentration-dependent (5×10^{-6}–1×10^{-4} M) positive inotropic and positive chronotropic responses in guinea-pig atrial tissues. The potency of B_{11} was greater than that of amrinone. The cardiotonic effects of B_{11} were not modified by β-adrenoceptor blockade. Carbachol inhibited the positive inotropic effect of B_{11}. The activity of B_{11} was increased in desensitized left atrial tissues. B_{11} inhibited the activities of PDE isoenzymes (type I, II, IV and V) from dog heart ventricle and PDE type IV from guinea-pig heart ventricle nonselectively.

It is concluded that B_{11} possesses potent positive inotropic activity in guinea-pig atria, and the effect is probably mediated by a non-selective inhibition of PDE activity.

Treatment of congestive heart failure is still a major therapeutic problem in clinical practice. New advances in the understanding of the pathophysiology, biochemistry and molecular biology of the disease has led to the search for new therapeutic agents that might influence the underlying pathology and slow the progression of myocardial damage, while at the same time improve patients' well-being (Yusuf et al 1986).

A wide range of cardiotonic agents which have both positive inotropic and vasodilator effects have been developed (Braunwald 1986), including amrinone and milrinone (Alousi & Johnson 1986), enoximone (Dage et al 1982), piroximone (Kariya et al 1984), imazodan (Bristol et al 1984), pimobendan (Fujimoto & Matsuda 1989) and saterinone (Armah et al 1988).

Our laboratory has been interested in finding compounds with cardiotonic and platelet aggregation inhibitory activity (Monge et al 1991a, b) and we have synthesized and studied the biological activity of a new series of 7,8-dimethoxy-pyrimido[5,4-*b*]indole derivatives.

In these compounds, we combine the nucleus of pyrimidine found in a variety of selective phosphodiesterase inhibitors with cardiotonic activity, such as buquineram, carbazeram and bemarinone, with the indole group related to inhibition of blood-platelet aggregation (Cross et al 1986). Some of these compounds exhibited notable properties as inhibitors of blood-platelet aggregation or inhibitors of the cyclic guanosine monophosphate (cGMP)-inhibited cAMP phosphodiesterase isolated from dog heart ventricles. Some of them possessed structural elements which could provide them with cardiotonic activity.

In this paper we report the results of the inotropic and chronotropic responses of guinea-pig isolated atrial tissues to 4-(4′-*n*-butylaniline)-7,8-dimethoxy-5H-pyrimido[5,4-*b*]indole (B_{11}, Fig. 1).

Materials and Methods

Isolated left and right atrial preparations

Male guinea-pigs, 250–400 g, were killed with a blow to the head and exsanguinated. The heart was excised and placed in a Petri dish filled with aerated Tyrode solution (for composition, see below), in which the left and right atria were dissected. The atria were suspended in 10-mL organ baths containing Tyrode solution composed of (mM): NaCl 136, KCl 5·4, $CaCl_2$ 1·8, $MgCl_2$ 1·05, NaH_2PO_4 0·42, $NaHCO_3$ 11·9 and glucose 5·5.

The solution was maintained at 34°C and gassed continuously with 95% O_2–5% CO_2. The tissues were

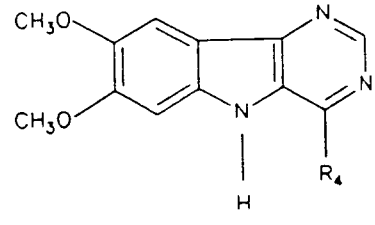

FIG. 1. Chemical structure of the 4-(4′-*n*-butylaniline)-7,8-dimethoxy-5H-pyrimido[5,4-*b*]indole.

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stretched by adjusting the diastolic tension to 1 g. Under these conditions the right atria beat spontaneously. The left atria were electrically stimulated at a basal rate of 1 Hz with square-wave pulses of 1 ms duration at a voltage 50% above threshold derived from a stimulator (Letica LI 12106, Barcelona, Spain). The frequency and amplitude of the contractions were recorded isometrically with a force-displacement transducer (Letica TRI 110, Barcelona, Spain) attached to a chart recorder through a preamplifier (Leitca PRS 206, Barcelona, Spain).

Drugs were added after an initial equilibration period of 30 min, during which time, control measurements of contractile force (left and right atria) and cardiac frequency (right atria) were recorded. The compound was first dissolved in dimethylsulphoxide (DMSO), then diluted in a buffer containing Tris HCl and albumin. According to this procedure, the maximum concentration of the vehicle used (0·5% DMSO, 0·002% albumin and 0·03% Tris HCl) did not produce appreciable inotropic and chronotropic effects.

We studied the effect of B_{11} at different concentrations with the aim of establishing a concentration-effect relationship. Each concentration of drug was added to the bath in a noncumulative manner. Only one concentration was assayed in each atrial tissue. Due to low solubility, the maximum concentration of B_{11} tested was 1×10^{-4} M. For this reason, it was not known whether the maximum response induced by this agent was reached at this concentration or whether concentrations above this would give meaningful results. The inotropic agent amrinone was used as the reference drug in the conditions described for the test compound.

In some experiments carried out to evaluate the effects of carbachol on the positive inotropic effect of B_{11}, carbachol (10 nM) was added for 30 min to left atrial tissues previously treated with B_{11} (5×10^{-6} M). Carbachol was applied when the inotropic response of B_{11} had reached the steady-state level. In these studies, isoprenaline (1×10^{-8} M) was used for comparison.

In another set of experiments, β-receptors were blocked with (±)-propranolol at 1×10^{-6} M for 30 min before the addition of B_{11} (1×10^{-4} M) to left and right atrial tissues and the inotropic and chronotropic response of the agent was determined in the presence of the β-blocker.

In a series of experiments, left atrial tissues were desensitized by incubating them for 60 min with a supramaximum concentration of isoprenaline (1×10^{-6} M). They were then stabilized for another 60 min (washed at 10-min intervals). Under these conditions, the tissues did not respond to the posterior addition of an effective concentration of the β-agonist. The inotropic effects of B_{11} and amrinone at a concentration of 1×10^{-4} M were measured after desensitizing the tissues.

The effects of the compounds were expressed as percentage change from control values (amplitude and frequency measurements made just before the addition of the drugs).

Determination of phosphodiesterase activity

cAMP-PDE from guinea-pig and dog heart muscle were purified according to the method of Reeves with minor modifications (Reeves et al 1987). PDE IV fraction from a DEAE-sepharose column was collected and dialysed against 2 L bis-Tris 20 mM/2-mercaptoethanol 5 mM/benzamidine 2 mM EDTA 2 mM and sodium acetate 50 mM. The dialysis solution was changed once after 5 h and then set aside overnight. The dialysed sample was applied to a DEAE-sepharose column (17 cm × 1·6 cm) pre-equilibrated with the same buffer, and eluted with 150 mL linear gradient of 0·35–1·0 M sodium acetate in the homogenization buffer.

For long-term storage, ethylene glycol was added to a final concentration of 30% (v/v) and fractions were stored at −20°C. Activity was stable for several weeks under these conditions.

PDE assay of cAMP and cGMP was carried out according to the procedure of Thomson et al (1979), except that specific activities of tritiated nucleotides (cAMP and cGMP) were 0·25 Ci mmol^{-1}.

For the evaluation of the inhibitory effects of B_{11} on the different isoenzymes, a concentration of 1×10^{-4} M was used. B_{11} was disolved in DMSO with the final concentration of DMSO in the reaction medium being 2·5%. B_{11} was also tested at different concentrations to estimate the IC50 (concentration of drug reducing the basal activity of PDE to 50%, by linear regression analysis) value for inhibition of type IV PDE isolated from dog heart myocardium. The inhibitory effects of amrinone were also studied for comparison.

Drugs

The following drugs were used: 4-(4′-*n*-butylaniline)-7,8-dimethoxy-5H-pyrimido[5,4-*b*]indole, synthesized in the Medicinal Chemistry Department (Centro de Investigación en Farmacobiología Aplicada, Universidad de Navarra, Spain), isoprenaline hydrochoride, (±)-propranolol hydrochloride and carbachol (Sigma, St Louis, MO); amrinone was kindly provided by Sterling-Winthrop Research Laboratories (Madrid, Spain); [8-^{3}H]adenosine 3′:5′-cyclic monophosphate, ammonium salt and [8-^{3}H]guanosine 3′:5′-cyclic monophosphate, ammonium salt (Amersham, Buckinghamshire, UK).

Statistical analysis

The results are expressed as mean ± s.e.m. Differences between mean values were determined with Student's *t*-test for paired data or Mann-Whitney U-test where appropriate. Values were considered to be statistically different when P was lower than 0·05. In the figures, vertical bars indicate ± s.e.m.

Results

Inotropic and chronotropic effects of compound B_{11}

Compound B_{11} produced a notable increase in force of contraction on both left and right atria and was accompanied by a parallel increase in cardiac frequency of right atria.

The addition of B_{11} at concentrations between 5×10^{-6} and 1×10^{-4} M in a noncumulative manner caused concentration-dependent increases in muscle developed tension. The onset of the inotropic response was within 1 min, while peak tension was reached after 5–10 min. This maximum effect was followed by a weak decline in the tension developed above baseline level during the 20 min in which

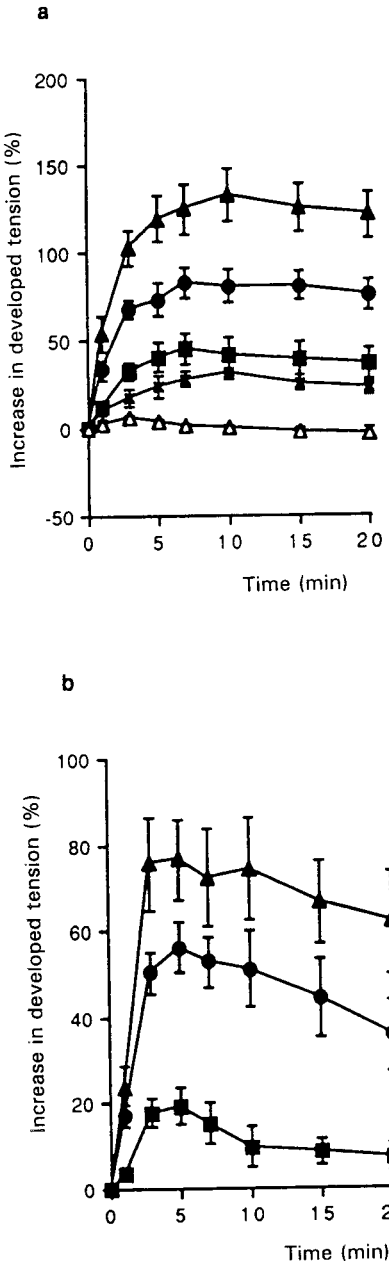

FIG. 2. Effect of (a) B_{11} and amrinone (10^{-4} M, ▲; 10^{-5} M, ■; 5×10^{-5} M, ●; vehicle (△) and 5×10^{-6} M, ×, and (b) amrinone (10^{-4} M, ■; 10^{-3} M ●; 3×10^{-3} M ▲ on electrically driven guinea-pig left atrial developed tension at various time intervals. Symbols represent the mean ± s.e.m. values (n = 5–8).

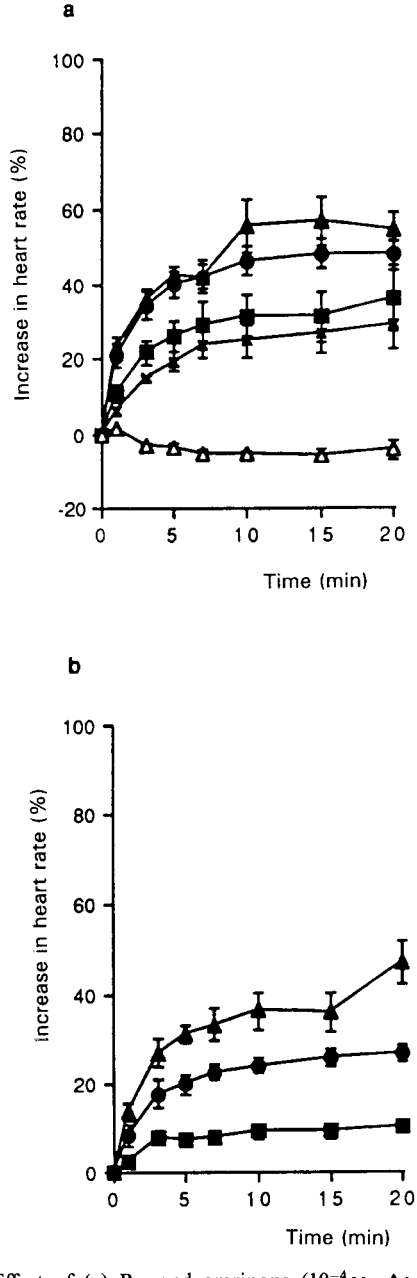

FIG. 3. Effect of (a) B_{11} and amrinone (10^{-4} M, ▲; 10^{-5} M, ■; 5×10^{-5} M, ●; 5×10^{-6} M ×; vehicle △) and (b) amrinone (10^{-4} M, ■; 10^{-3} M, ●; 3×10^{-3} M, ▲) on rate of spontaneously beating right atria at various time intervals. Symbols represent the mean ± s.e.m. values (n = 5–8).

the test lasted (Fig. 2a). B_{11} did not produce any tachyarrhythmias or contractures.

Likewise, B_{11} produced a positive chronotropic effect on guinea-pig right atria, which followed a parallel course to the inotropic effect. The onset of the chronotropic effect appeared in the first minute and gradually increased reaching a maximum between 10 and 15 min after the addition of the drug and maintained itself there during the 20 min of the assay. This increase in the cardiac frequency was concentration-dependent (Fig. 3a).

Amrinone added to the bath in a noncumulative manner (1×10^{-4}, 1×10^{-3} and 3×10^{-3} M) showed positive inotropic effects in guinea-pig left and right atria, accompanied by an increase of heart beat in the right auricle. The effect on the contraction force in stimulated left auricle was concentration-dependent and reached a maximum at 3 to 5 min after the addition of the drug, decreasing progressively with time (Fig. 2b).

In right atria, the addition of amrinone produced an increase in the contractile force in the same way and reached its maximum after 3 min. This effect is accompanied by an important increase in the cardiac frequency in a concentration-dependent manner, which was observed

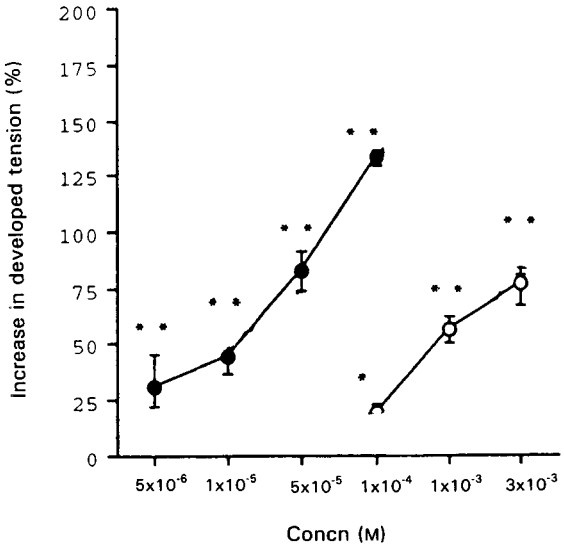

FIG. 4. Effect of B_{11} (●) and amrinone (○) on left atrial developed tension. Symbols represent the mean ± s.e.m. values (n = 5–8) of maximum percentage changes in contractile tension. * $P<0.05$ and ** $P<0.01$ significantly different from control values (Student's paired t-test).

immediately after the addition of the drug. The effect increased progressively and reached a maximum 20 min after the drug was added (Fig. 3b).

At a concentration of 1×10^{-4} M the percentage increases in force of contraction in left and right atria for B_{11} were 132·8 ± 14·9 and 117·9 ± 9·3%, respectively, whereas for amrinone, they were less (19·3 ± 4·2 and 15·1 ± 2·0%) respectively. The effect of B_{11} on the inotropic response appeared at concentrations lower than those for amrinone. Therefore, the concentration–response curve for B_{11} moved towards the left with respect to the curve for amrinone (Fig. 4).

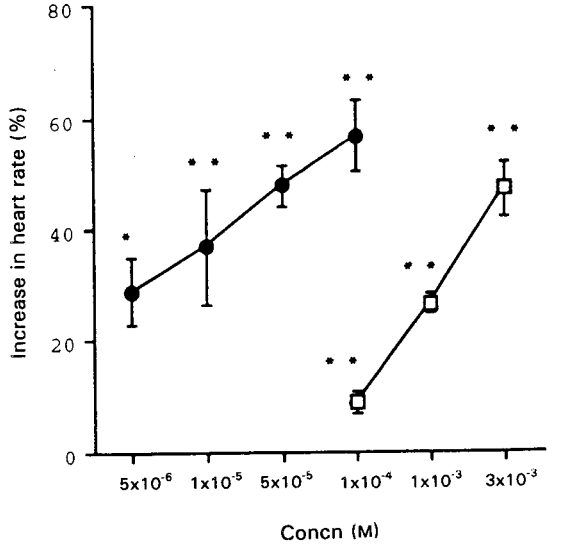

FIG. 5. Chronotropic effect of B_{11} (●) and amrinone (□) on spontaneously beating right guinea-pig atria. Symbols represent the mean ± s.e.m. values (n = 5–8) and are maximum percentage changes in beats min^{-1} from basal values. * $P<0.05$ and ** $P<0.01$ significantly different from control values (Student's paired t-test).

Table 1. Effect of propranolol on the positive inotropic and chronotropic effect of B_{11} in electrically driven left atria and spontaneously beating right atria.

	Inotropic effect (left atria)	Chronotropic effect (right atria)
Basal	890·7 ± 128·2	168 ± 7
+ B_{11}	2004·3 ± 240·3	244 ± 21
Basal	850·7 ± 134·0	179 ± 9
+ propranolol	555·3 ± 103·9	144 ± 11
+ propranolol + B_{11}	1478·8 ± 196·4	195 ± 22

Data represent maximum isometric tension (mg) and chronotropic activity (beats min^{-1}). Each result is mean ± s.e.m. of four to six assays from different experiments.

In spontaneous-beating right atria, both compounds exerted a positive chronotropic effect. The maximal increase in chronotropic action under B_{11} treatment was 56·4 ± 6·4% at an end-concentration of 1×10^{-4} M. At the same concentration amrinone produced only a 9·0 ± 1·9% increase over basal values. However, at an end-concentration of 3×10^{-3} M which produced a 76·6 ± 9·5% increase in left atrial developed tension, the percentage increase was of 46·9 ± 4·8% (Fig. 5).

Effect of β-adrenoceptor blockade

Incubation of right and left atrial tissues in 1×10^{-6} M (±)-propranolol for 60 min resulted in a complete blockade of the inotropic and chronotropic responses of the tissues to 1×10^{-9}–1×10^{-8} M isoprenaline (data not shown). Under these experimental conditions, pre-incubation of the organs with propranolol did not affect the inotropic and chronotropic response of B_{11} at the concentration of 1×10^{-4} M. Although a slight decrease in the chronotropic response is observed, it is not significant (Table 1).

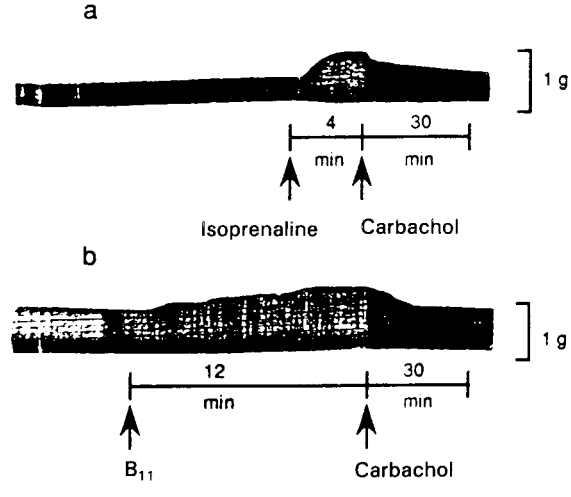

FIG. 6. Original recording showing the effect of carbachol 10 nM on the contractile activity of isoprenaline (a) and B_{11} (b) in electrically driven left atria (n = 4).

Table 2. Inotropic effect of B_{11} and amrinone in normal and desensitized electrically driven left guinea-pig atria.

Drug	Non-desensitized		Desensitized		
	Basal	+Drug	Basal	+Isoprenaline	+Isoprenaline +drug
B_{11}	920 ± 156	2140 ± 255	854 ± 125	354 ± 106	2145 ± 327
Amrinone	882 ± 198	1060 ± 164	768 ± 156	275 ± 89	975 ± 205

Data represent maximum isometric tension (mg). Each result is the mean ± s.e.m. of four to six assays from different experiments.

Influence of carbachol on the positive inotropic effect of B_{11}
The addition of carbachol (10 nM) to electrically stimulated left atria, does not modify the basal activity of the tissue. In the same concentration, carbachol completely reversed the positive inotropic response induced by B_{11} during the steady-state level of contraction.

Isoprenaline, an agent which increases cAMP levels, has been used in these studies and a similar result has been observed after the addition of carbachol.

Effects of carbachol on the positive inotropic effects induced by B_{11} and isoprenaline are shown in Fig. 6.

Inotropic effect of B_{11} in desensitized guinea-pig left atria
Exposure to isoprenaline for 60 min resulted in an approximately 60–66% decrease in contractile force after washing the tissue, and in a blunting of the responsiveness to the β-agonist (data not shown).

The results indicated in Table 2 show that B_{11} not only maintained its inotropic activity effectively in these tissues but also that the percentage increase in the developed tension was greater than that found in non-desensitized atria. A similar effect was observed with the reference compound amrinone.

Effects on phosphodiesterase activity
Four main peaks of PDE activity were obtained by ion-exchange chromatography from dog heart ventricles. According to Beavo & Reifsnyder (1990), the peaks were designated as follows. Peak I (PDE type I) is a Ca^{2+}/calmodulin cyclic nucleotide PDE, peak II (type II) is a cGMP-stimulated cyclic nucleotide PDE, peak III (type III) is a cGMP-inhibited PDE and peak IV (type IV) is a cAMP-specific PDE.

Four peaks of PDE from guinea-pig hearts could also be resolved by ion-exchange chromatography, although only the inhibition of PDE type III was determined.

As shown in Table 3, B_{11} (1×10^{-4} M) inhibited PDE type I, II, III and IV activities isolated from dog ventricular myocardium and PDE type III activity of guinea-pig ventricles. Thus, B_{11} is a nonselective inhibitor of PDE. The % inhibition of PDE III activity in dog and guinea-pig ventricular myocardium were similar or identical. This behaviour is observed among other compounds tested in our laboratories and is in concordance with other authors (Schmitz et al 1989). The effects of amrinone were also examined. As indicated in Table 3, amrinone is a selective inhibitor of type III PDE, although the inhibition of type IV PDE is also notable at the concentration assayed. On the other hand, the inhibition of type I and type III PDE is negligible. There is also a good correlation between the inhibiton of type III PDE activity isolated from dog or guinea-pig myocardium.

The IC50 value for inhibition of type III PDE activity obtained from dog ventricles had been calculated for both compounds, being 28·8 mM for B_{11} and 47·0 mM for amrinone.

Discussion

In the present study, the cardiotonic activity of 4-(4′-*n*-butylaniline)-7,8-dimethoxy-5H-pyrimido[5,4-*b*]indole was evaluated.

In guinea-pig isolated left and right atria, B_{11} caused a concentration-dependent positive inotropic effect which was accompanied by positive chronotropic action. Likewise, amrinone produced concentration-dependent inotropic and chronotropic effects. In these studies B_{11} seemed to be more potent than the reference compound, amrinone.

Since propranolol did not affect the observed responses in both atria, neither the inotropic nor the chronotropic effect was mediated by activation of β-adrenoceptors.

Continuous exposure to a drug or hormone results in a

Table 3. Percentage inhibition of the cAMP-PDE activities of dog and guinea-pig ventricular myocardium by B_{11} and amrinone at a concentration of 1×10^{-4} M.

Compound	% Inhibition				
	Dog heart PDE				Guinea-pig heart PDE
	Type I	Type II	Type III	Type IV	Type III
B_{11}	49·8 ± 5·6	61·2 ± 1·0	62·9 ± 2·0	66·3 ± 4·0	61·9 ± 4·9
Amrinone	−12·7 ± 9·9	3·1 ± 5·5	57 ± 1·5	47·5 ± 3·4	53·3 ± 4·0

loss or diminution of the responsiveness of the exposed tissue or cell to that same drug or hormone, and this is the case for β-adrenergic receptor-mediated responses (Harden 1983).

Cardiac β-agonist-induced desensitization of the response to catecholamines is a well-known phenomenon (Marsh et al 1979, 1982; Chang et al 1982). Similarly, in chronic heart failure, there is a reduced inotropic response to β-adrenergic stimulation which has been demonstrated in-vitro and in-vivo (Brodde 1991). This is caused by exposure to increased amounts of neurotransmitter due to the activation of the sympathetic nervous system (Bristow et al 1988).

In the present study the prolonged exposure to the β-agonist isoprenaline in guinea-pig isolated left atria induced desensitization of the response to the following addition of this agent. Although this treatment can not necessarily be regarded as a mode of human heart failure, there is a decreased cardiac responsiveness to the β-agonist, similar to that found in failing human hearts. In fact, in failing human hearts, the inotropic effects of PDE inhibitors are blunted (Feldman et al 1987; Schmitz et al 1989; Steinfath et al 1992), when compared with their effects in healthy human myocardium or in animal heart muscle preparations from healthy laboratory animals.

In this study, we do not known the molecular mechanism underlying β-agonist-induced desensitization (receptor downregulation, altered amounts or function of G-proteins (Barnett 1989; Bristow et al 1990; Feldman & Bristow 1990; Horn & Bilezikian 1990), but as amrinone greatly increased its inotropic response compared with that observed in non-desensitized tissues, it is reasonable to assume that in this preparation there is no enhanced breakdown or insufficient rise in cAMP. The present finding that the positive inotropic effect of compound B_{11} is also enhanced in these tissues, supports the hypothesis that its activity is not mediated by β-adrenoceptor stimulation.

In spite of the fact that compound B_{11} presented a similar behaviour to that of amrinone, we can not conclude that the inotropic responses induced by both agents are mediated by the same mechanism of action.

Carbachol antagonized the positive inotropic effect of B_{11}. Carbachol is known to inhibit cAMP-dependent response by lowering cAMP in different heart preparations (e.g., elevated by adenylate cyclase stimulation of PDE inhibition) (Endoh 1979; Endoh et al 1982, 1985) as can be observed from the inhibition of the cAMP-mediated inotropic response to isoprenaline.

Amrinone is generally referred to as an inhibitor of so-called cGMP-inhibited cAMP-PDE (Kariya & Dage 1988). Although recent data indicate that other mechanisms may be responsible for its inotropic and chronotropic responses, such as an antagonism towards endogenous adenosine (Dorigo & Maragno 1986) and stimulation of adenylate cyclase by functional block of the inhibitory guanine nucleotide regulatory protein, Gi (Parson et al 1988), it is the increase in cAMP levels which is implied in its inotropic and chronotropic actions (Shahid & Rodger 1989; Muller et al 1990).

The effect of carbachol on the inotropic response of compound B_{11} offers indirect evidence for the cAMP involvement in the effect of this compound. Furthermore, the effects of B_{11} on the activities of the different PDE isoenzymes (Type I, II, III and IV) in homogenates from dog heart ventricles showed that B_{11} potently inhibited the activities of the four forms of PDE to a similar extent. Thus compound B_{11} proved to be a nonselective PDE inhibitor. Nevertheless, the selectivity for PDE III inhibition does not appear to be a prerequisite for a drug to exhibit a positive inotropic effect (Schmitz et al 1989) and both selective (e.g. amrinone, milrinone, enoximone, adibendan, saterinone) and nonselective (theophylline, trapidil) PDE inhibitors increase cardiac force of contraction.

In conclusion, the results of the present study show that the new synthesized 7,8-dimethoxy-5H-pyrimido[5,4-*b*]indole derivative, B_{11}, possesses potent positive inotropic activity in guinea-pig isolated atria. This effect is probably mediated by a nonselective inhibition of PDE activity.

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References

Alousi, A. A., Johnson, D. C. (1986) Pharmacology of the bipyridine: amrinone and milrinone. Circulation 73 (Suppl. III): 10–24

Armah, B. I., Hofferber, E., Jacobitz, P. (1988) Positive inotropic and vasodilatory actions of saterinone in vivo. Arzneim. Forsch. 38: 1303–1309

Barnett, D. B. (1989) Myocardial β-adrenoceptor function and regulation in heart failure: implications for therapy. Br. J. Clin. Pharmacol. 27: 527–537

Beavo, J. A., Reifsnyder, D. H. (1990) Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. Trends Pharmacol. Sci. 11: 150–155

Braunwald, E. (1986) Newer positive inotropic agents. Circulation 73 (Suppl. III): 1–3

Bristol, J. A., Sircar, I., Moos, W. H., Evans, D. B., Weishaar, R. E. (1984) Cardiotonic agents. 1. 4,5-Dihydro-6-[4-(1H-imidazol-1-yl)phenyl]-3(2H)-pyridazinones. Novel positive inotropic agents for the treatment of congestive heart failure. J. Med. Chem. 27: 1099–1101

Bristow, M. R., Sandoval, A. B., Gilbert, E. M., Deisher, T., Minobe, W., Rasmussen, R. (1988) Myocardial α- and β-adrenergic receptors in heart failure: is cardiac-derived norepinephrine the regulatory signal? Eur. Heart. J. 9 (Suppl. H): 35–40

Bristow, M. R., Hershberger, R. E., Port, J. D., Gilbert, E. M., Sandoval, A., Rasmussen, R., Cates, A. E., Feldman, A. M. (1990) β-Adrenergic pathways in nonfailing and failing human ventricular myocardium. Circulation 82 (Suppl. I): I12–I25

Brodde, O.-E. (1991) β_1- and β_2-Adrenoceptors in the human heart: properties, function and alterations in chronic heart failure. Pharmacol. Rev. 43: 204–242

Cross, P. E., Dickinson, R. P., Parry, M. J., Randall, M. J. (1986) Selective thromboxane synthetase inhibitors. 2. 3-(1H-Imidazol-1-ylmethyl)-2-methyl-1H-indole-1-propanoic acid and analogues. J. Med. Chem. 29: 342–346

Chang, M. Y., Klein, R. M., Kunox, G. (1982) Selective desensitization of cardiac beta-adrenoceptors by prolonged in vivo infusion of catecholamines in rats. J. Pharmacol. Exp. Ther. 221: 784–789

Dage, R. C., Roebel, L. E., Hsiech, C. P., Weiner, D. L., Woodward, J. K. (1982) Cardiovascular properties of a new cardiotonic agent: MDL 17043. J. Cardiovasc. Pharmacol. 4: 500–508

Dorigo, P., Maragno, J. (1986) Interaction of amrinone with endogenous adenosine in guinea pig atria. Br. J. Pharmacol. 87: 623–629

Endoh, M. (1979) Correlation of cyclic AMP and cyclic GMP levels with changes in contractile force of dog ventricular myocardium during cholinergic antagonism of positive actions of histamine, glucagon, theophylline and papaverine. Jpn. J. Pharmacol. 29: 855–864

Endoh, M., Yamashita, S., Taira, N. (1982) Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. J. Pharmacol. Exp. Ther. 221: 775–783

Endoh, M., Yanagisawa, T., Morita, T., Taira, N. (1985) Differential effects of sulmazole (Ar-L115 BS) on contractile force and cyclic AMP levels in canine ventricular muscle: comparison with 17043. J. Pharmacol. Exp. Ther. 234: 267–273

Feldman, A. M., Bristow, M. R. (1990) The β-adrenergic pathway in the failing human heart: implications for inotropic therapy. Cardiology 77 (Suppl. 1): 1-32

Feldman, A. M., Copelas, L., Gwathmey, J. K., Phillips, P., Warren, S. E., Schoen, E. I., Grossman, W., Morgan, J. P. (1987) Deficient production of cyclic AMP: pharmacologic evidence of an important cause of contractile dysfunction in patients with end-stage heart failure. Circulation 75: 331–339

Fujimoto, S., Matsuda, T. (1989) Effects of pimobendan, a cardiotonic and vasodilating agent with phosphodiesterase inhibiting properties, on isolated arteries and veins of rats. J. Pharmacol. Exp. Ther. 252: 1304–1311

Harden, T. K. (1983) Agonist-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase. Pharmacol. Rev. 35: 5–32

Horn, E. M., Bilezikian, P. (1990) Mechanisms of abnormal transmembrane signaling of the β-adrenergic receptor in congestive heart failure. Circulation 82 (Suppl. I): I26–I34

Kariya, T., Dage, R. C. (1988) Tissue distribution and selective inhibition of subtypes of high affinity cAMP phosphodiesterase. Biochem. Pharmacol. 37: 3267–3270

Kariya, T., Wille, L. J., Dage, R. C. (1984) Studies on the mechanism of the cardiotonic activity of MDL 19205: effects on several biochemical systems. J. Cardiovasc. Pharmacol. 6: 50–55

Marsh, J. D., Barry, W. H., Neer, E. J., Alexander, R. W., Smith, T. W. (1979) Desensitization of chick embryoventricle to the physiological and biochemical effects of isoproterenol. Circ. Res. 45: 528–540

Marsh, J. D., Barry, W. H., Smith, T. W. (1982) Desensitization to the inotropic effect of isoproterenol in cultured ventricular cells. J. Pharmacol. Exp. Ther. 223: 60–67

Monge, A., Aldana, I., Alvarez, T., Font, M., Santiago, E., Latre, J. A., Bermejillo, M. J., Lopez-Unzu, M. J. (1991a) New 5H-pyridazino [4,5,*b*]indole derivatives. Synthesis and studies as inhibitors of blood platelet aggregation and inotropics. J. Med. Chem. 34: 3023–3029

Monge, A., Aldana, I., Alvarez, T., Losa, M. J., Font, M., Cenarruzabeitia, E., Lasheras, B., Frechilla, D., Castiella, E., Fernández-Alvarez, E. (1991b) 1-Hydrazino-4-(3,5-dimethyl-1-pyrazolyl) -5H-pyridazino [4,5-*b*]indole. A new antihypertensive agent. Eur. J. Med. Chem. 26: 655–658

Muller, B. C., Lugnier, C., Stoclet, J. C. (1990) Implication of cyclic AMP in the positive inotropic effects of cyclic GMP-inhibited cyclic AMP phosphodiesterase inhibitors on guinea pig isolated left atria. J. Cardiovasc. Pharmacol. 15: 444–451

Parson, W. J., Ramkumar, V., Stiles, G. L. (1988) The new cardiotonic agent sulmazole is an A_1 adenosine receptor antagonist and functionally blocks the inhibitory regular Gi. Mol. Pharmacol. 33: 441–448

Reeves, M. L., Leigh, B. K., England, P. J. (1987) The identification of a new cyclic nucleotide phosphodiesterase activity in human and guinea pig cardiac ventricle. Biochem. J. 241: 531–541

Schmitz, W., Von der Leyen, H., Meyer, W., Neumann, J., Scholz, H. (1989) Phosphodiesterase inhibition and positive inotropic effects. J. Cardiovasc. Pharmacol. 14 (Suppl. 3): S11–S14

Shahid, M., Rodger, I. W. (1989) Chronotropic and inotropic actions of amrinone, carbazeran and isobutylmethylxanthine: role of phosphodiesterase inhibition. Br. J. Pharmacol. 98: 291–301

Steinfath, M., Danielsen, W., Von der Leyen, H., Mende, U., Meyer, W., Neumann, J., Nose, M., Reich, T., Schmitz, W., Scholz, H., Starbatty, J., Stein, B., Döring, V., Kalmar, P., Haverich, A. (1992) Reduced α_1- and β_2-adrenoceptor-mediated positive inotropic effects in human end-stage heart failure. Br. J. Pharmacol. 105: 463–469

Thomson, W. J., Terasaki, W. L., Epstein, P. M., Strada, S. J. (1979) Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of enzyme. Adv. Cyclic Nucl. Res. 10: 69–91

Yusuf, S., Wittes, J., Bailey, K., Furberg, C. (1986) Digitalis—a new controversy regarding an old drug. The pitfalls of inappropriate methods. Circulation 73: 14–18