# Effects of Isoprenaline on Tonic Tension and Na-Ca Exchange in Frog Atrial Fibres

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**Abstract.** The action of isoprenaline, a purely $\beta$-agonist, was investigated on frog atrial fibres under voltage clamp conditions; tonic tension was induced by long depolarizing pulses and the outward delayed current simultaneously developed. The cumulative dose-response curves showed that isoprenaline increased the peak of tonic tension in the concentration range $10^{-8}$ to $3 . 10^{-6}$ mol . $l^{-1}$, with a maximum effect for $10^{-6}$ mol . $l^{-1}$. The positive inotropic action of isoprenaline was associated with an increase in the rates of tension rise and of relaxation. Isoprenaline also increased the amplitude of the outward delayed current in a dose-dependent manner. The effects of isoprenaline ($10^{-6}$ mol . $l^{-1}$) on tonic tension and outward delayed current were not observed in the presence of propranolol ($10^{-7}$ mol . $l^{-1}$). Experiments carried out in low-sodium solution demonstrated that the action of isoprenaline on tonic tension can be explained by activation of Na-Ca exchange; the enhanced relaxation might result from the same process. These results suggested that the positive inotropic action of isoprenaline is mediated not only by the well-known increase in the slow inward current but also by activation of the Na-Ca exchange mechanism.

**Key words**: Atrial heart muscle — Tonic tension — Na/Ca exchange — Isoprenaline

## Introduction

In Amphibia, where adrenaline rather than noradrenaline is the neurotransmitter (Falck et al. 1963; Azuma et al. 1965), cardiac adrenoceptors are predominantly $\beta_2$-receptors (Benfey 1977; Stene-Larsen and Helle 1978); stimulation of these receptors changes the time course of the action potential and contraction (Antoni et al. 1960; Graham and Lamb 1966; Brady 1967). Studies performed on frog atrial fibres using the double voltage-clamp technique have demonstrated the electrophysiological and mechanical effects of catecholamines (Vassort et al. 1969; Driot et al. 1970; Morad et al. 1981; Soustre and Rakotonirina 1981). $\beta$-adrenergic stimulation was shown to increase the amplitude and dura-

tion of the plateau of the action potential and the duration of the action potential; an increase in peak tension was associated with an increase in both the rate of tension development and the time-to-peak tension. Such β-adrenergic responses are specific for the amphibian heart (Soustre and Rakotonirina 1981). The relaxation of tension is also classically enhanced.

In the frog heart, extracellular calcium plays an evident role in the regulation of contractility. The slow inward current during the plateau of the action potential contributes directly to the development of the phasic component of contraction (Einwächter et al. 1972; Léoty and Raymond 1972; Vassort and Rougier 1972; Horackova and Vassort 1976), and the net calcium influx by the Na-Ca exhange mechanism regulates the tonic component of contraction (Goto et al. 1971; Vassort 1973; Chapman 1974; Benninger et al. 1976; Miller and Moisescu 1976; Horackova and Vassort 1979).

The positive inotropic action of β-catecholamines through the activation of the slow inward current is now well documented on amphibian cardiac preparations (Vassort et al. 1969; Driot et al. 1970; Goto et al. 1980; Soustre and Rakotonirina 1981; Ouedraogo et al. 1982), but the effects of β-agonists on tonic tension and Na-Ca exchange have not been investigated in depth.

The present study on frog atrium was undertaken to test the action of isoprenaline, as a function of its concentration, on tonic tension and to determine the influence of this purely β-agonist on Na-Ca exhange.

## Materials and Methods

### *Preparation*

The experiments were performed at room temperature (18—20 °C) on trabeculae (100—150 μm in diameter and 3 or 4 mm in length) isolated from frog (*Rana esculenta* or *Rana ridibunda*) auricle. The preparations were mounted in a double mannitol-gap apparatus as described by Léoty and Alix (1976); the electrical responses were recorded from the central test compartment under voltage clamp conditions. The tension generated by the portion of the trabeculae in the test gap was measured with a variable resistance transducer (AE 801 Akers Electronics) as described previously (Soustre and Rakotonirina 1981).

### *Solutions*

The control Ringer solution used as the bathing and perfusing medium had the following composition (mmol.$l^{-1}$): NaCl 100; KCl 2.5; $CaCl_2$ 2; $MgCl_2$ 2; Hepes (N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid) 10; the pH was adjusted to 7.8 with NaOH. In some solutions, the sodium concentration was lowered to 70 mmol.$l^{-1}$ by replacing NaCl with an equimolar amount of LiCl. Tetrodoxin ($1.5 \times 10^{-6}$ mol.$l^{-1}$) and $MnCl_2$ (3 to $4.5 \times 10^{-3}$ mol.$l^{-1}$) were added to all solutions as fast and slow inward current inhibitors; $LaCl_3$ ($10^{-3}$ mol.$l^{-1}$) was used as Na-Ca exchange inhibitor. Isotonic mannitol (220 mmol.$l^{-1}$) was used as insulating solution.

The $\beta$-agonist used in the present work was ($\pm$) isoprenaline hydrochloride (Sigma). The stock solution of isoprenaline was prepared in 1 % ascorbic acid solution and kept at 0 °C to prevent oxidative degradation. ($\pm$) Propranolol was used as $\beta$-receptor antagonist.

*Procedure*

The atrial preparations were stimulated at a frequency of 0.1 Hz; depolarizing pulses were applied from the holding potential, which was defined as zero reference. The holding potential was adjusted until the amplitude of the fast inward current elicited by a 40 mV step depolarization reached its maximum value ($h_\infty \simeq 1$). Tonic tension was induced by long depolarizations (> 450 ms); during the depolarization an outward delayed current also developed, but there was no correlation between tonic tension and this outward current. The peak of tonic tension and the maximal rates of tension development and relaxation were measured. The amplitude of the outward delayed current was determined as the difference between the total outward current measured at the end of the depolarizing pulse and the leakage current measured at the beginning of the pulse.

In order to construct cumulative dose-response curves for isoprenaline action on membrane current and tension, each preparation was perfused with successively increasing concentrations of the drug in Ringer solution; for any concentration, current and tension were recorded when a steady level was reached (3 min). The observations were carried out on five preprations and the results were statistically analyzed using Student's *t*-test. A P-value equal to or les than 0.05 was considered to represent significant differences.

Experiments were done to test the effects of isoprenaline on the Na-Ca exchange mechanism using a procedure described previously (Soustre et al. 1986). A reduction of the external sodium concentration induces a transient increase in peak tonic tension, which is the result of activation of the Na-Ca exchange: Ca influx linked to Na efflux is enhanced while Ca efflux linked to Na influx is inhibited (Horackova and Vassort 1979). The transient increase in peak tonic tension was taken as an index of Na-Ca exchange activity. Each curve illustrated is representative of four to six experiments.

## Results

### 1 —*Effects of isoprenaline on the delayed outward current and tonic tension*

#### a) Dose-response curves

The delayed outward current and tonic tension were elicited by long depolarizations (about 600 ms) of 130 mV; the responses of five frog atrial fibres to various isoprenaline doses were studied.

The records obtained from one preparation of the series (Fig. 1*A*) show that isoprenaline at $10^{-7}$ mol.$l^{-1}$ increased the amplitude of the delayed outward current and the peak of tonic tension; the rates of tension development and relaxation were also enhanced. The effects of isoprenaline were more pronounced at $10^{-6}$ mol.$l^{-1}$.

The results are summarized in Table 1. The effects of isoprenaline on the outward delayed current and on tonic tension were concentration-dependent:

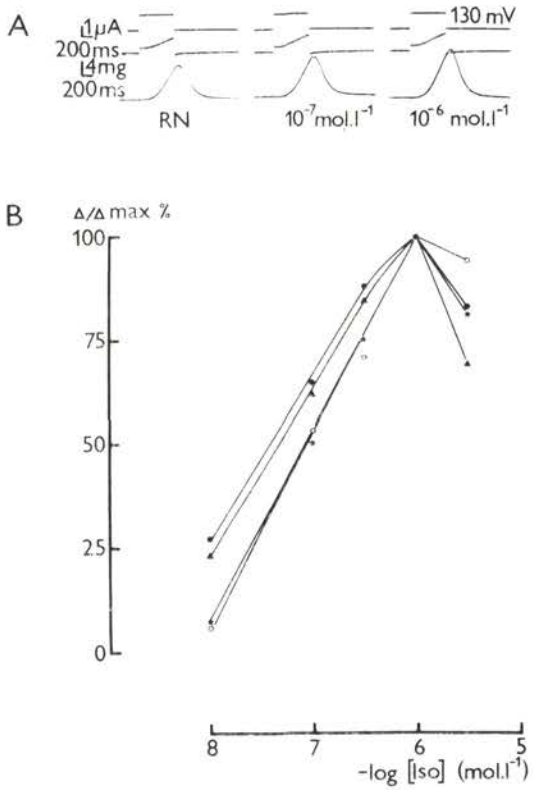


**Fig. 1.** *A*: Effects of two concentrations of isoprenaline on the outward delayed current (*middle trace*) and tonic tension (*lower trace*) induced by a 130 mV clamp step (*upper trace*). *B*: Concentration-response curves for isoprenaline effects on the outward delayed current ($I_x$; ○), the peak of tonic tension ($T$; ●) and the maximal rates of tension development ($V_T$; ▲) and of relaxation ($V_R$; ★). The mean variation of each parameter is expressed as percent of the maximal variation.

the amplitude of the outward current ($I_x$), the peak of tonic tension ($T$), and the maximal rates of contraction ($V_T$) and relaxation ($V_R$) progressively increased upon raising isoprenaline concentration from $10^{-8}$ mol.$l^{-1}$ to $10^{-6}$ mol.$l^{-1}$. At $3.10^{-6}$ mol.$l^{-1}$, the effectiveness of isoprenaline was reduced, suggesting a possible toxic effect of this drug concentration. The positive inotropic action of isoprenaline seems to result from the increase in the rate of tension development. For any concentration, the increase in amplitude of the outward delayed current is insignificant, but the variations of all the tension parameters are highly significant for $3.10^{-7}$ and $10^{-6}$ mol.$l^{-1}$. The dose-response curves are shown in Fig. 1*B*; the mean variations of each parameter, expressed as percentage of the

**Table 1.** Influence of increasing concentrations of isoprenaline on the amplitude of the outward delayed current ($I_x$), the peak of tonic tension ($T$) and the maximal rates of tension rise ($V_T$) and relaxation ($V_R$); $n = 5$. Mean value ± standard error; $^*P \leqslant 0.05$.

| Parameters | Control | Isoprenaline (mol/l) | | | | |
|---|---|---|---|---|---|---|
| | | $10^{-8}$ | $10^{-7}$ | $3 \times 10^{-7}$ | $10^{-6}$ | $3 \times 10^{-6}$ |
| $I_x$ ($\mu$A) | 0.70 ± 0.13 | 0.71 ± 0.11 | 0.79 ± 0.10 | 0.82 ± 0.10 | 0.87 ± 0.10 | 0.86 ± 0.14 |
| $T$ (mg) | 4.81 ± 0.93 | 5.99 ± 1.73 | 7.63 ± 1.61 | 8.67* ± 1.36 | 9.19* ± 1.38 | 8.44 ± 1.42 |
| $V_T$ (g/s$^{-1}$) | 0.010 ± 0.002 | 0.013 ± 0.003 | 0.018 ± 0.003 | 0.021* ± 0.003 | 0.023* ± 0.003 | 0.019* ± 0.003 |
| $V_R$ (g/s$^{-1}$) | 0.011 ± 0.002 | 0.013 ± 0.003 | 0.019* ± 0.001 | 0.023* ± 0.002 | 0.027* ± 0.003 | 0.024* ± 0.004 |

maximal variation, are plotted as a function of isoprenaline concentration. The curves confirm the dose-dependent pattern of isoprenaline action.

b) Current-voltage and tension-voltage relationships

The effects of a single dose of isoprenaline ($10^{-6}$ mol.l$^{-1}$) on the outward delayed current and on tonic tension were investigated by determinating current-voltage and tension-voltage relationships. The preparations, perfused with TTX-Mn-Ringer solution, were stimulated by different potentials; current and tension were recorded in control solution and 3 min after the addition of the catecholamine (Fig. 2).For any potential, isoprenaline increased both the delayed outward current and tonic tension, with the effects being obvious for large depolarizations.

c) Influence of propranolol on the effects of isoprenaline

In order to determine whether the $\beta$-adrenoceptors are implicated in the effects of isoprenaline the influence of a $\beta$-adrenoceptor blocking agent, propranolol was investigated. Propranolol was present in Ringer solution throughout the experiment and 7 min before the perfusion with isoprenaline ($10^{-6}$ mol.l$^{-1}$); three concentrations of propranolol were used: $3.10^{-8}$, $10^{-7}$ and $10^{-6}$ mol.l$^{-1}$ (Table 2). In our experimental conditions, the delayed outward current and

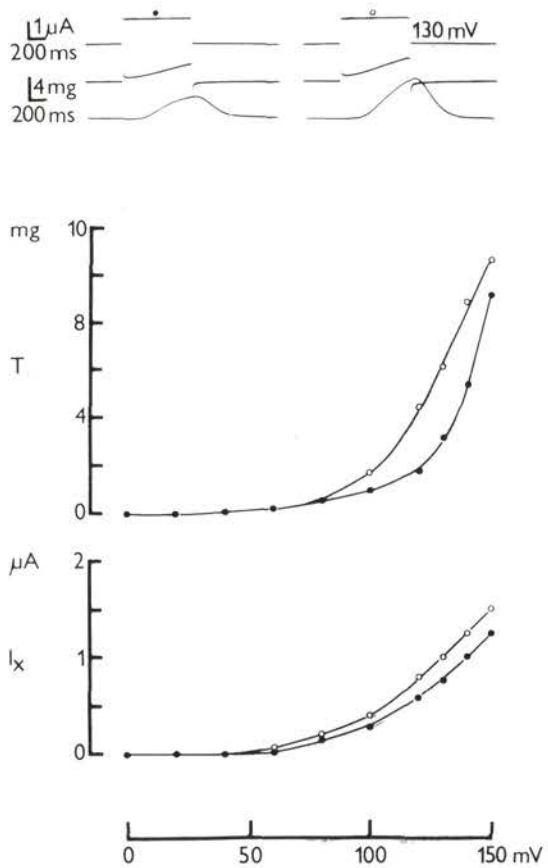


**Fig. 2.** Current-voltage and tension-voltage relationships for the outward delayed current ($I_x$) and tonic tension ($T$) in the absence (•) and in the presence of isoprenaline, $10^{-6}$ mol.$l^{-1}$ (○). *Top*: Examples of current and tension recorded for 130 mV depolarization in control solution (•) and after isoprenaline (○).

tonic tension were slightly decreased by any propranolol concentration used. The positive inotropic effect of isoprenaline was not antagonized by the lowest dose of propranolol $3.10^{-8}$ mol.$l^{-1}$, and in the presence of $10^{-7}$ and $10^{-6}$ mol.$l^{-1}$ propranolol isoprenaline had no significant inotropic effect. The isoprenaline-induced increase in the delayed outward current was less important in the presence of $3.10^{-8}$ mol.$l^{-1}$ and $10^{-7}$ mol.$l^{-1}$ propranolol than in the absence of the $\beta$-blocker; with $10^{-6}$ mol.$l^{-1}$ propranolol plus isoprenaline, the delayed outward current was somewhat decreased. It may be noted that all the current changes are insignificant. Figure 3 presents records obtained from one prepra-

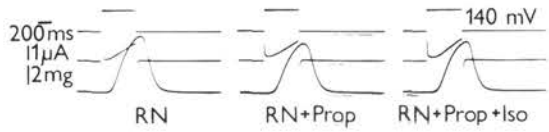


**Fig. 3.** Influence of a $\beta$-adrenoceptor blocking agent on the action of isoprenaline; outward delayed current (middle trace) and tonic tension (lower trace) elicited by a 140 mV depolarization are successively recorded in control solution (RN), after perfusion with propranolol $10^{-7}$ mol.$l^{-1}$ (RN + Prop.) and after exposure to isoprenaline ($10^{-6}$ mol.$l^{-1}$) in the presence of propranolol (RN + Prop + Iso).

**Table 2.** Influence of pretreatment with different doses of propranolol (Prop.) on the action of isoprenaline (ISO) on the amplitude of the outward delayed current ($I_x$) and tonic tension ($T$). Mean value ± standard error; $* P \leqslant 0.05$.

| | | $T$ (mg) | $I_x$ ($\mu$A) |
|---|---|---|---|
| I | Control | 6.71 ± 1.08 | 1.53 ± 0.10 |
| ($n = 4$) | Prop. $3 \times 10^{-8}$ mol/l | 5.78 ± 1.08 | 1.34 ± 0.08 |
| | Prop. + Iso $10^{-6}$ mol/l | *9.19 ± 1.64 | 1.54 ± 0.13 |
| II | Control | 6.60 ± 0.59 | 1.19 ± 0.14 |
| ($n = 6$) | Prop. $10^{-7}$ mol/l | 6.46 ± 0.95 | 1.17 ± 0.17 |
| | Prop. + Iso $10^{-6}$ mol/l | 6.44 ± 0.81 | 1.27 ± 0.17 |
| III | Control | 5.64 ± 0.76 | 0.87 ± 0.10 |
| ($n = 4$) | Prop. $10^{-6}$ mol/l | 5.28 ± 0.72 | 0.83 ± 0.07 |
| | Prop. + Iso $10^{-6}$ mol/l | 5.50 ± 0.80 | 0.78 ± 0.07 |

tion of series II: after propranolol perfusion, the leakage current was slightly increased and tonic tension decreased; at this point isoprenaline caused a quite insignificant increase in the outward delayed current and tonic tension.

## 2 — *Effects of isoprenaline on the Na-Ca exchange system*

### a) Effects of isoprenaline on tonic tension in normal and low-sodium solution

The transient increase in tonic tension induced by a reduced external sodium concentration gives an estimate of Na-Ca exchange activity. In these experiments it was chosen to lower the external sodium concentration to 70 mmol.$l^{-1}$ to induce a moderate transient increase in tonic tension. In some experiment, the

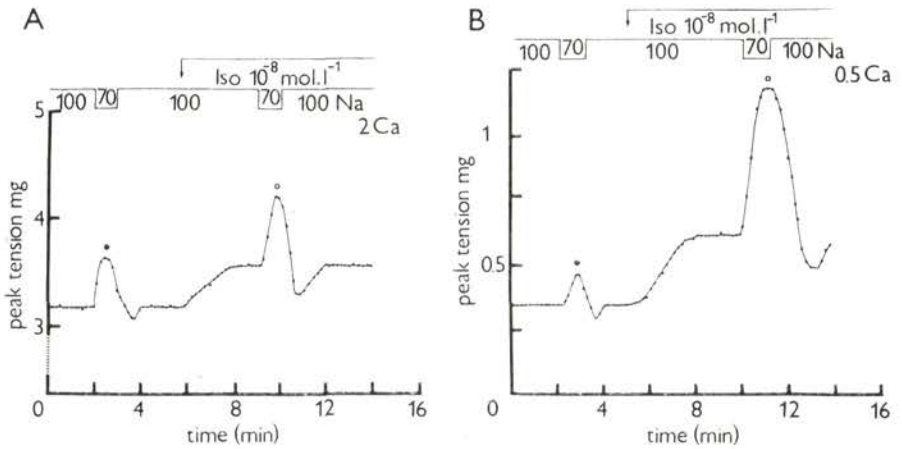


**Fig. 4.** Effects of a low-sodium solution (70 mmol/l) on peak tonic tension in the absence (•) and in the presence (○) of isoprenaline; calcium concentration was 2 mmol.$l^{-1}$ (*A*) and 0.5 mmol.$l^{-1}$ (*B*).

Ringer solution contained only 0.5 mmol.$l^{-1}$ $Ca^{2+}$, so that the initial tonic tension was weakly developed, and a low dose of isoprenaline ($10^{-8}$ mol.$l^{-1}$) was used. The purpose was to avoid saturation of the contractile proteins with too high concentration of intracellular $Ca^{2+}$ in the presence of isoprenaline and in a low sodium solution.

As shown in Figure 5*A*, lowering the external sodium to 70 mmol.$l^{-1}$ induced a transient increase in peak tension (about 14 % over the control level). After the return of tonic tension to the initial level in normal Ringer solution, the preparation was perfused with isoprenaline; tonic tension increased to a new steady value and after switching to the low-sodium solution, a somewhat greater transient increase in tonic tension was observed (17 %).

In low-calcium Ringer's (Fig. 5*B*), the tension transiently increased (by about 33 % over the control value) upon lowering external sodium to 70 mmol.$l^{-1}$. In the presence of isoprenaline, tonic tension increased; a subsequent reduction of external sodium to 70 mmol.$l^{-1}$ induced an important transient increase in tonic tension (94 %).

b) Influence of lanthanum on the effect of isoprenaline on tonic tension

The influence of lanthanum ($LaCl_3$), a Na-Ca exchange inhibitor, on the effect of isoprenaline on tonic tension was tested. Lanthanum was shown to inhibit the exchange current on frog atrial trabeculae at the concentration of $2.10^{-3}$

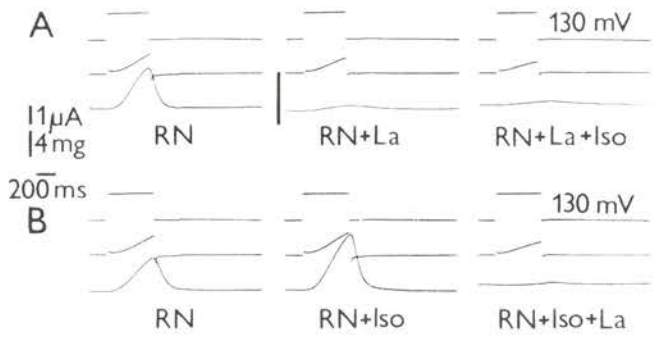


**Fig. 5.** Influence of lanthanum (La, $10^{-3}$ mol.$l^{-1}$) on the action of isoprenaline ($3 \times 10^{-7}$ mol.$l^{-1}$) on the outward delayed current (middle trace) and tonic tension (lower trace) induced by a 130 mV clamp step. $LaCl_3$ was added to Ringer solution (RN) either 5 min before isoprenaline (*A*) or after 3 min of perfusion with isoprenaline (*B*). In *A*, note the different tension scale in the presence of lanthanum.

mol.$l^{-1}$ (Mentrard et al. 1984). Lanthanum concentrations ranging from $3.10^{-4}$ mol.$l^{-1}$ to $2.10^{-3}$ mol.$l^{-1}$ induced a concentration-dependent decrease in tonic tension, which was fully inhibited by $2.10^{-3}$ mol.$l^{-1}$ lanthanum (not shown). Figure 5 shown the effect of $10^{-3}$ mol.$l^{-1}$ lanthanum on the response to $3.10^{-7}$ mol.$l^{-1}$ isoprenaline. Lanthanum added to normal Ringer solution depressed tonic tension to about 7% of the control value within 5 min; when isoprenaline was applied in the presence of lanthanum the tonic tension remained inhibited (Fig. 5*A*). Moreover, the positive inotropic effect of isoprenaline was inhibited by lanthanum (Fig. 5*B*). Also, an inhibitory effect of lanthanum on the outward delayed current was observed.

## Discussion

The present study showed that on frog atrium isoprenaline evidently increases the tonic component of tension. Goto et al. (1980) reported isoprenaline ($10^{-6}$ mol.$l^{-1}$) to decrease rather than increase tonic tension on bullfrog atrium; however their records have been not convincing. Classical pharmacological dose-response relationships suggest a dose-dependent action of isoprenaline: tonic tension is enhanced at concentrations between $10^{-8}$ and $3.10^{-6}$ mol.$l^{-1}$,

with the maximal effect being obtained at $10^{-6}$ mol.$l^{-1}$. Similar isoprenaline concentrations enhanced the $I_{si}$-dependent phasic component of tension in our experiments (not shown). Moreover, it was reported in a previous work that isoprenaline, within the same range of concentrations, induced a dose-dependent increase in contraction of frog atrial fibres recorded simultaneously with the action potential in current clamp conditions (Soustre and Rakotonirina 1981).

In the presence of isoprenaline, the outward delayed current which contributes to repolarization of the action potential increased in a dose-dependent manner; however, this effect was insignificant for any concentration. Similar findings were reported when $\beta$-adrenoceptor stimulation was induced by epinine, a biogenic catecholamine (Soustre and Rakotonirina 1981). An increase in the outward delayed current was observed in frog atrium with a single concentration of $\beta$-agonists also by other investigators (Goto et al. 1980; Umeno 1984), but in isolated frog atrial cells concentrations of isoprenaline which produced a large increase in the slow inward current were shown to have no detectable effect on the delayed outward current (Hume 1985).

In the present work, propranolol ($10^{-7}$ mol.$l^{-1}$) was effective in blocking the effects of isoprenaline on tonic tension and the outward delayed current; similar concentrations of propranolol were shown to block the action of adrenaline (Goto et al. 1980) and isoprenaline (Umeno 1984) on the outward delayed current in bullfrog atrium. Thus, the action of isoprenaline on both tonic tension and the outward delayed current seems to depend on $\beta$-adrenoceptor stimulation.

Tonic tension induced by a long depolarization ($> 250$ ms) is related to the Na-Ca exchange mechanism; Ca influx linked to Na efflux contributes to an increase in cytoplasmic free calcium and generates tonic tension. The transient increase in tonic tension elicited by a low-sodium solution was used to evaluate the Na-Ca exchange activity. Suitable experimental conditions were defined in order to avoid Ca-saturation of the contractile system when applying low-sodium solution in the presence of isoprenaline.

The transient increase in tonic tension in low sodium solution was more accentuated in the presence of isoprenaline: Ca influx linked to Na efflux was enhanced. Accordingly, in normal sodium solution, the isoprenaline-induced increase in tonic tension can be explained by activation of the Na-Ca exchange mechanism.

The intracellular sodium content is an important factor in the modulation of intracellular calcium and tonic tension through the Na-Ca exchange mechanism (Horackova and Vassort 1979; Chapman and Tunstall 1983; Horackova 1985). It was shown that intracellular sodium activity ($aNa_i$) is higher in frog heart than in mammalian heart (Chapman and Tunstall (1984), and during the

increase in tension induced by lowering external sodium the intracellular calcium content rises to reach relatively high values while $aNa_i$ does not significantly fall (Chapman 1983). Although data on the effect of isoprenaline on $aNa_i$ in frog heart are not available it may be assumed that isoprenaline increases calcium influx and tonic tension without a significant fall in $aNa_i$ in this tissue as well; moreover, $aNa_i$ may remain high because of the isoprenaline-mediated increase in Na entry via the slow channel (Driot et al. 1970).

Lanthanum is known to inhibit Na-Ca exchange in squid axons (Baker 1972) and cardiac sarcolemmal vesicles (Trosper and Philipson 1983) as well as the exchange current in frog atrial fibres (Mentrard et al. 1984). Although lanthanum is not a selective inhibitor of Na-Ca exchange (Kass and Tsien 1975; Roulet et al. 1979; Bielefeld et al. 1986; Kennedy et al. 1986), it was prefered to other Na-Ca exchange inhibitors such as verapamil and D-600 (Horackova 1983) since the latter drugs may enter the cells and act intracellularly (Entman et al. 1972; Fabiato and Fabiato 1973; Vaghy et al. 1982). Moreover, amiloride shown to inhibit Na-Ca exchange in cardiac preparations (Siegl et al. 1984; Kennedy et al. 1986; Debetto et al. 1987), may be a potent antagonist of $\beta$-adrenoceptors (Häussinger et al. 1987). The Na-Ca exchange-dependent tonic tension was effectively inhibited by lanthanum and the isoprenaline-mediated increase in tonic tension was also abolished by this agent. These results support the view that isoprenaline affects the Na-Ca exchange mechanism.

From the present observations, that the isoprenaline-induced increase in tonic tension is mediated by $\beta$-adrenoceptor stimulation it may be postulated that the increase in Na-Ca exchange activity is also due to the action on $\beta$-adrenoceptors.

It is generally admitted that in the frog heart Na-Ca exchange alone could be sufficient to induce relaxation without a need for Ca-sequestration into the sarcoplasmic reticulum or Ca extrusion by the ATP-dependent Ca-pump (Goto et al. 1972; Kavaler and Anderson 1978; Chapman 1979; Roulet et al. 1979). The mitochondria do not seem to play a significant role in relaxation of myofibrils in frog heart (Scarpa and Graziotti 1973). Na-dependent mitochondrial accumulation of Ca may occur only when $aNa_i$ is lowered, e.g. during Na-withdrawal contracture or during inhibition of Na pump (Chapman 1983). In the presence of $\beta$-agonist, the enhanced rate of relaxation of tonic tension at the end of depolarization may result from an increase in Na-Ca exchange, which at resting potential corresponds to Ca efflux linked to Na influx. The relaxation of phasic tension is accelerated in the presence of isoprenaline: this classical effect of $\beta$-stimulation (Morad et al. 1978; Goto et al. 1980) may also be explained by an enhancement of the Na-Ca exchange mechanism. Nevertheless, it was shown in amphibian heart that C-protein phosphorylation in response to $\beta$-adrenergic agonist plays a role in regulating the relaxation rate (Hartzell 1984).

In conclusion, β-catecholamines stimulate the transport of Ca via Na-Ca exchange during membrane depolarization, resulting in an increase in the tonic component of contraction. This process, also linked to the well-known increase in $I_{si}$-dependent phasic tension, contributes to the positive inotropic action of β-agonists. These drugs also enhance the rate of relaxation, due to activation of the Na-Ca exchange-dependent Ca efflux. It may be suggested that β-agonist potentiate both Ca entry during systole and Ca efflux during diastole by regulating phosphorylation of the Na-Ca exchange protein. c AMP-dependent protein phosphorylation is widely accepted as the general mechanism by which intracellular events respond to β-adrenergic stimulation. However the role of cAMP in mediating the relaxant effect of β-agonists has not been obvious (Morad et al. 1981) and no effect of cAMP-dependent protein kinase on the Na-Ca exchange system could be demonstrated (Rinaldi et al. 1982). Moreover, the Na-Ca exchange mechanism may be subject to $Ca^{2+}$-calmodulin-dependent phosphorylation (Caroni and Carafoli 1983). Further investigations are required to determine the biochemical events involved in the activation of the Na-Ca exchange by β-adrenoceptor stimulation.

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