brief communication

A novel action of isoproterenol to inactivate a cardiac K^+ current is not blocked by beta and alpha adrenergic blockers

Cinzia Tromba and Ira S. Cohen
Department of Physiology and Biophysics, State University of New York, Stony Brook, New York 11794 USA

ABSTRACT The K^+ current i_{KI} sets the resting potential in cardiac cells. Here we report that isoproterenol (ISO), a prototypical β agonist, increases inactivation of i_{KI}. This action of ISO on i_{KI} is mimicked by permeant analogues of cAMP but is not blocked by the β blockers propranolol and pindolol or the α blockers prazosin or yohimbine. We suggest that this novel action of ISO may contribute to pacemaker activity in the Purkinje strand and be mediated through a class of receptors different from classical β's or α's.

INTRODUCTION

Isoproterenol is a potent β agonist which increases both the strength and frequency of cardiac contractions. These effects of isoproterenol are largely mediated through increases in calcium current and pacemaker current (1–3). The changes in these two membrane currents occur through the activation of β adrenoceptors, because both are blocked by the β blocker propranolol. Additional actions of isoproterenol on cardiac K^+ currents include an increase in delayed rectifier current, an increase in background K^+ conductance, and a change in the kinetics of inactivation of the transient outward current (4–7). These actions of isoproterenol also involve the activation of β adrenoceptors and also are blocked by propranolol. We report below a novel action of isoproterenol to inactivate the inwardly rectifying K^+ conductance, i_{KI}, in canine cardiac Purkinje myocytes. This action of isoproterenol to inactivate i_{KI} is mimicked by permeant analogues of cAMP which shift the relationship between inactivation of i_{KI} and membrane potential in the positive direction along the voltage axis. Surprisingly, this action of isoproterenol is not blocked by propranolol up to 10 μM, nor is it blocked by the α_1 blocker prazosin, or the α_2 blocker yohimbine. This novel action of isoproterenol is of interest because it presents evidence for modulation of i_{KI}, an important cardiac potassium conductance. These results also suggest that a novel mechanism may couple isoproterenol to intracellular levels of cAMP. A presentation of some of this work was made to the British Physiological Society (8).

Address offprint requests to Ira S. Cohen, Dept. of Physiology and Biophysics, Health Science Center, SUNY at Stony Brook, Stony Brook, NY 11794-8661.

METHODS

Our experiments were performed on isolated canine Purkinje myocytes acutely dissociated from the Purkinje fibers as previously described (9–11). We employed the whole cell voltage clamp technique with pipettes ranging in resistance from 1.5 to 3 MΩ before sealing. The currents were recorded with the axopatch 2A amplifier in most cases with series resistance compensation. The pipette was filled with a solution containing in millimolar: KCl 140, NaCl 10, $MgCl_2$ 2, $CaCl_2$ 1, EGTA 11, ATP 1, GTP 0.1, and Hepes KOH 10, all buffered to a final pH of 7.2. The superfusing Tyrode solution contained in millimolar: NaCl 140, KCl 5.4 or 10.0, $MgCl_2$ 1.8, $CaCl_2$ 0.5 or 1.5, glucose 5.5, and Hepes NaOH 5, all buffered to a final pH of 7.4. The temperature at which the experiments were carried out was 35 ± 1°C. Stock solutions of isoproterenol, propranolol, prazosin, and yohimbine were prepared in distilled water and subsequently diluted in the perfusate. 500 μM/liter ascorbic acid was added to the stock solution containing isoproterenol to prevent oxidation. Dibutyryl cAMP (dBcAMP) and chlorophenylthiocAMP (CPTcAMP) were directly dissolved in the saline at the concentrations indicated.

RESULTS

Fig. 1 *a* illustrates our basic finding. When a canine Purkinje myocyte is hyperpolarized from a holding potential of −50 mV to a potential negative to E_k there is large increase of inward current due to activation of the inwardly rectifying K^+ current i_{KI}. In the presence of isoproterenol (ISO) 1 μM the same hyperpolarization results in the activation of i_{KI} which is followed by a pronounced inactivation. The enhanced inactivation of i_{KI} is readily reversed upon washout of ISO. ISO caused increased inactivation of i_{KI} in just over 60% (24 of 39) of cells studied.

ISO is known to alter many cardiac conductances by stimulating adenylyl-cyclase and elevating the basal levels of cAMP (12). We decided to examine whether the inactivation of i_{KI} was also mediated by cAMP. To investigate this alternative we employed two permeable

0006-3495/90/09/791/05 $2.00

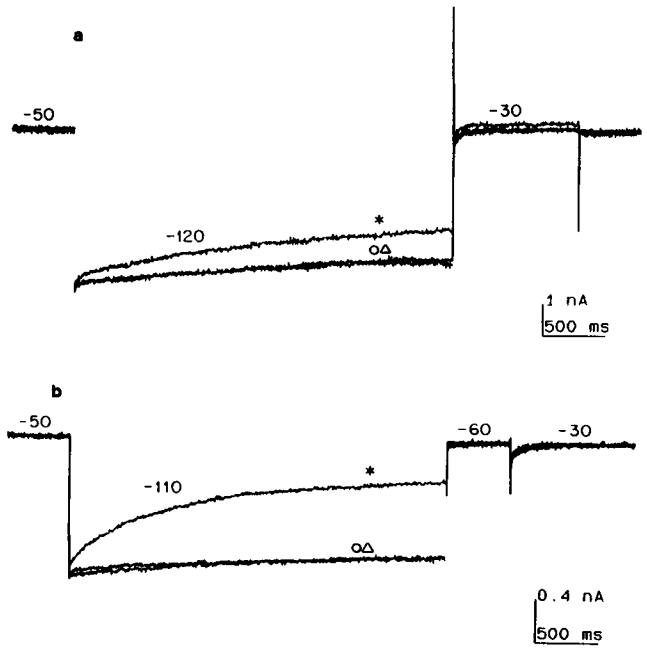

FIGURE 1 ISO and cAMP analogues enhance i_{K1} inactivation. (*a*) Traces recorded before (o), during (*), and after (Δ) perfusion with 1 μM ISO. K_o = 10 mM. (*b*) Effect of perfusion of CPTcAMP 1 mM. K_o = 5.4 mM. The symbols have the same meaning as in *a*. Exp. s 025-1, 302-1.

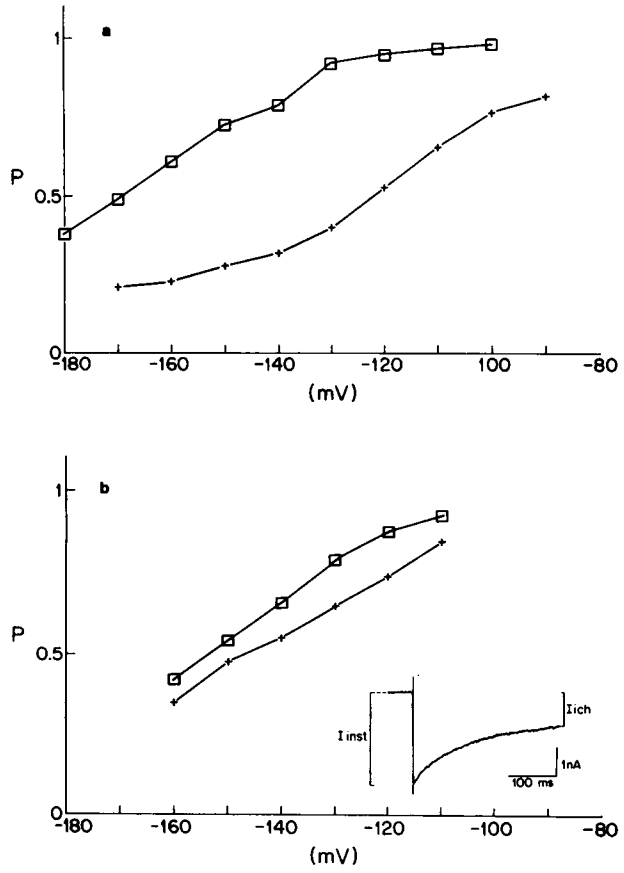

FIGURE 2 Inactivation curves measured in the presence and in the absence of cAMP analogues at different external K concentrations. The method used to construct the curves is illustrated in the inset. From brief (200–300 ms) hyperpolarizing pulses, we estimated the value of instantaneous current by fitting the decay of i_{K1} with a single or double exponential, and then we extrapolated the fitted curve to zero time. *P* represents the ratio of the value of the current at the end of the pulse (isochronal, i_{ich}) to the extrapolated instantaneous current (i_{inst}) and provides an estimation of the degree of i_{K1} inactivation at different voltages. Notice that cAMP analogues cause the curves to move in a positive direction along the voltage axis and that the amount of this shift is greater at lower K_o concentrations. (*a*) K_o = 5.4 mM; Exp. 005-4. (*b*) K_o = 10 mM; Exp. 004-6. (□) Control; (+) 0.5 mMdBcAMP. We performed this experiment in a total of eight cells, four bathed in 5.4 mM and four in 10 mM K^+, and we used either dBcAMP or CPTcAMP at concentrations ranging between 0.5 and 2 mM/liter. Under these conditions we obtained an average shift of the inactivation curve of 33 mV in 5.4 K^+ and 16 mV in 10 mM K^+.

analogues of cAMP, dibutyryl cAMP (dBcAMP, 0.5–2 mM), and cholorophenylthiocAMP (CPTcAMP, 0.5–1 mM), which were dissolved in our superfusing solution. Both analogues of cAMP were effective in inducing inactivation of i_{K1}, quite similar to that obtained with ISO (Fig. 1 *a*). An example of these results is given in Fig. 1 *b*, where CPTcAMP 1 mM was employed. Permeable analogues of cAMP increased inactivation of i_{K1} in 70% (31 of 44) of cells studied.

Recent investigations of i_{K1} in ventricular myocytes have demonstrated that inactivation of i_{K1} is voltage dependent, being far more prominent at more hyperpolarized potentials (13, 14). ISO is known to shift the gating of at least one cardiac membrane current which activates on hyperpolarization, i_f, to more positive potentials on the voltage axis (2, 3). We therefore examined whether the increased inactivation of i_{K1} corresponded to a positive shift of the inactivation versus voltage curve on the voltage axis. The protocol we employed was first reported by Biermans et al. (14) and assumes that the dominant conductance change responsible for the jump in inward current is i_{K1}. We confirmed this assumption by applying 3–5 mM Ba^{2+}, a blocker of i_{K1}, which reduced the inward jump on hyperpolarization from −40 to −120 by >80% in four cells. Also, no time-dependent increase in outward current was observed in Ba^{2+}-Tyrode, confirming that the inactivation process we observed involved i_{K1}. Our results examining the relationship between inactivation and voltage are illustrated in Fig. 2. We employed Tyrode's solution containing either 10 or 5.4 mM K^+ and compared the control solution with that containing dBcAMP at a concentration of 0.5 mM/liter. There was a large

positive shift of the relationship between inactivation and voltage for both solutions, however, the half inactivation level was shifted further in 5.4 than in 10 mM K^+ (see legend to Fig. 2).

We next examined the receptor type through which ISO exerted its effects. Our initial hypothesis was that the inactivation of i_{KI} was exerted via classical β adrenoceptors, because ISO is a prototypical β agonist and cAMP is the known second messenger through which β activation exerts its effects (15). To test this hypothesis we applied 1 μM propranolol which blocks all classical cardiac β adrenoceptors. One sample result is illustrated in Fig. 3 *a*. In this experiment we employed a two-pulse protocol: first, a hyperpolarization to observe i_{KI} followed after a brief pulse to -60 mV by a depolarization to observe the calcium current. We employed this protocol to demonstrate the difference between the effect of ISO on i_{KI} and that on other cardiac membrane currents. On application of ISO, there is an increase in the instantaneous jump in current on hyperpolarization. This was sometimes observed and may correspond to the increase in background K^+ conductance previously reported by Gadsby (6). This conductance change is thought not to be mediated through i_{KI}. ISO also induces the inactivation of i_{KI} which we have illustrated in Fig. 1 *a*. On depolarization ISO induces the well-known increase in the magnitude of the calcium current. Next propranolol (1 μM) was added to the perfusing medium. It is clear that propranolol was able to reverse the effect on the background K^+ conductance as well as the increase in Ca^{2+} current, but not the inactivation of i_{KI}. This concentration of propranolol or lower has been shown to reverse other effects of β agonists on cardiac membrane currents (16, 17). Essentially the same result was obtained in the presence of a higher concentration of propranolol (10 μM) or when pindolol (1 μM) was substituted for propranolol (data not shown). It seems therefore unlikely that the ISO effect on i_{KI} is mediated through either β_1 or β_2 adrenoceptors.

Because α activation is known to reduce a background K^+ conductance in Purkinje myocytes (9), the next step we undertook was to check whether ISO was exerting its effect through α adrenoceptors. A typical result in this series of experiments is shown in Fig. 3 *b*. In this case ISO was applied in the presence of the α blockers prazosin (α_1, 1 μM) and yohimbine (α_2, 0.1 μM), as well as the β blocker propranolol (1 μM). We did not find substantial differences in another experiment where prazosin and yohimbine were used at concentrations of, respectively, 2 and 1 μM. Prazosin at 1 μM is known to block the phenylephrine-induced decrease in background K^+ conductance in Purkinje myocytes (9). We chose the highest concentration of the α_2 blocker yohimbine without direct membrane effects 10^{-7} M (18) and then also tried a concentration 10-fold higher, 1 μM. There is little information on effective concentrations for α_2 antagonism in heart, but in human platelets where most α receptors are α_2, isoproterenol is a very poor agonist and the concentrations of yohimbine we employed would block binding of isoproterenol to the receptor (19). It is evident from this data that even in a circumstance in which all the known α and β adrenoceptors should be blocked by their antagonists the ISO-induced increase in i_{KI} inactivation could not be reversed.

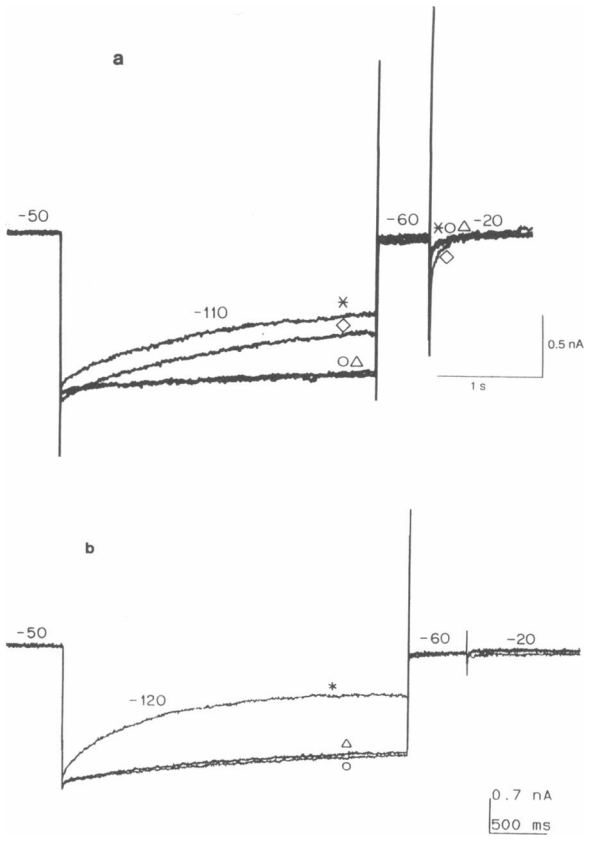

FIGURE 3 Neither classical α or β receptor antagonists are able to block ISO action on i_{K1}. (*a*) A two-pulse protocol was used to activate i_{Ca} in addition to i_{K1}. Propranolol (1 μM) is able to reverse the ISO-induced increase of i_{Ca} and background non-i_{K1} K current (7) but not the inactivation of i_{K1}. (O) Control, (◊) ISO 1 μM, (*) ISO 1 μM + propranolol 1 μM, (△) recovery. Exp. 302-10. (*b*) ISO effect on i_{K1} persists when α blockers are added in the presence of β blockers. (O) Control, (*) ISO 1 μM + propranolol 1 μM + prazosin 1 μM + yohimbine 0.1 μM, (△) recovery. Exp. 321-1. In another experiment (321-2) before testing ISO we perfused the cells with solutions containing only the α or β antagonists for up to 4 min, and detected no effects of any sort. Afterwards, we proceeded with a protocol as the one reported above and found analogous results.

DISCUSSION

The results presented above are significant for two reasons. First, we have demonstrated that the background conductance i_{K1} is subject to modulation. Second, some evidence is given supporting the idea that this regulation initiated by the prototypical β agonist ISO may not occur via β_1 or β_2 adrenoceptors. We will discuss each of these findings in turn.

ISO increases calcium current, pacemaker current, the K^+-selective delayed rectifier, and a non-i_{K1} K conductance. It also induces a novel inward current at plateau potentials (20–22). Our report presents the evidence for a direct action of this hormone on the background conductance i_{K1}. It is also the first definitive demonstration of modulation of i_{K1}. The shift in the inactivation versus voltage curve mediated by cAMP is less pronounced in 10 mM K^+ than it is in 5.4 mM K^+. This larger shift at physiologic K^+ implies that i_{K1} may inactivate positive as well as negative to E_K. If i_{K1} inactivated positive to E_K, it would produce a slow, time-dependent decrease in outward current. This time-dependent decrease in outward current would sum with the inward background current, and the hyperpolarization activated inward i_f to produce a net inward current. The net inward current would drive the membrane toward threshold. Thus, if i_{K1} inactivated positive to E_K, the Purkinje pacemaker rate would increase.

Neither β nor α antagonists were effective in blocking the inactivation of i_{K1} induced by ISO. This is surprising for two reasons. First, ISO is the classic β agonist, and second, cAMP is the second messenger of β activation. Sometimes we found the blockers increased the inactivation of i_{K1}. Occasionally, propranolol or pindolol could also lead to a similar time-dependent decrease of the current.

cAMP is known to be the second messenger for the adrenergic stimulation of i_{Ca} and its permeant analogues have been shown here to be able to mimic the ISO effect on i_{K1}. In this context, the inability of propranolol to reverse the effect of ISO on i_{K1} while fully antagonizing the drug action on i_{Ca} is puzzling. Our data so far do not enable us to answer this question, but we can suggest two possible explanations. Either, the two channels may exhibit different sensitivities to cAMP concentration, or cAMP compartmentalization may exist. If compartmentalization exists, it would mean that activation of normal β adrenoceptors would not give rise to a sufficient increase in [cAMP] locally to activate our response. High concentrations of permeant analogues of cAMP might, however, reach the relevant compartment. Furthermore, having used permeant analogues of cAMP dissolved in the external solution, we cannot even completely rule out the possibility that these substances act at a different site than ISO on the cell membrane, yet produce analogous effects.

Recently the existence of "atypical" β adrenoceptors has been reported. These receptors exist in various tissue types (adipocytes, smooth muscle cells, heart) (23–25). Binding and functional studies of this new class of receptors have demonstrated that they respond differently than β_1 and β_2 adrenoceptors to either β antagonists and/or partial agonists. In fact, they show a 10- to 100-fold decreased sensitivity to propranolol. In the heart it has been demonstrated that nonconventional partial β agonists such as pindolol give rise to a biphasic response whose low-sensitivity component is not blocked by specific β_1/β_2 antagonists (23–27). In adipocytes coupling of the atypical β adrenoceptor to adenylyl-cyclase (28) has been demonstrated. Furthermore, a gene has been recently isolated that may encode for this atypical β_3 adrenoceptor (29). In this same study the authors present data from transfection experiments that demonstrate that the product of this gene is coupled to adenylyl-cyclase and exhibits low affinity to standard β blockers.

Although we have not even demonstrated that an external receptor is required, and indeed many compounds inactivate ion currents by directly blocking channels (30, 31), the possibility exists that our novel effect of ISO on i_{K1} reported above may be mediated through this new class of adrenoceptors.

Although much remains to be examined, it is clear that the background K^+ conductance i_{K1} in the Purkinje myocyte is hormonally controlled and that isoproterenol may act on the heart in a manner other than classical β adrenoceptors activation.

We would like to thank Drs. Peter Pennefather, Michael Rosen, and Andrea Volterra for their helpful comments on the manuscript and Mrs. Joan Zuckermann for preparing the cells.

This work was supported by National Institutes of Health grants HL20558 and HL28958.

Received for publication 1 February 1990 and in final form 18 May 1990.

REFERENCES

1. Brum, G., W. Osterrieder, and W. Trautwein. 1984. β-Adrenergic increase in the calcium conductance of cardiac myocytes studied with the patch clamp. *Pfluegers Arch. Eur. J. Physiol.* 401:111–118.
2. Tsien, R. W. 1974. Effects of epinephrine on the pacemaker potassium current of cardiac Purkinje fibers. *J. Gen Physiol.* 64:293–319.
3. DiFrancesco, D. 1986. Characterization of single pacemaker channels in cardiac sino-atrial node cells. *Nature (Lond.).* 324:470–473.

4. Giles, W., T. Nakajima, K. Ono, and E. F. Shibata. 1989. Modulation of the delayed rectifier K^+ current by isoprenaline in bull-frog atrial myocytes. *J. Physiol. (Lond.)*. 415:233–249.
5. Duchatelle-Gourdon, I., H. C. Hartzell, and A. Lagrutta. 1989. Modulation of the delayed rectifier potassium current in frog cardiomyocytes by β-adrenergic agonists and magnesium. *J. Physiol. (Lond.)*. 415:251–274.
6. Gadsby, D. C. 1983. β-Adrenoceptor agonists increase membrane K^+ conductance in cardiac Purkinje fibres. *Nature (Lond.)*. 306:691–693.
7. Nakayama, T., C. Palfrey, and H. A. Fozzard. 1989. Modulation of the cardiac transient outward current by catecholamines. *J. Mol. Cell. Cardiol.* 21(Suppl.):109–118.
8. Cohen, I., and C. Tromba. 1989. Inactivation of i_{K1} in isolated Purkinje myocytes is induced by isoprenaline but not blocked by propranolol. *J. Physiol. (Lond.)*. 418:41P.
9. Shah, A., I. S. Cohen, and M. R. Rosen. 1988. Stimulation of cardiac alpha receptors increases Na/K pump current and decreases g_K via a pertussis toxin-sensitive pathway. *Biophys. J.* 54:219–225.
10. Cohen, I. S., N. B. Datyner, G. A. Gintant, N. K. Mulrine, and P. Pennefather. 1987. Properties of an electrogenic sodium-potassium pump in isolated canine Purkinje myocytes. *J. Physiol. (Lond.)*. 383:251–267.
11. Chang, F., J. Gao, C. Tromba, I. Cohen, and D. DiFrancesco. 1990. Acetylcholine reverses effects of β-agonists on pacemaker current in canine cardiac Purkinje fibers but has no direct action. A difference between primary and secondary pacemakers. *Circ. Res.* 66:633–636.
12. Tsien, R. W., W. Giles, and P. Greengard. 1972. Cyclic AMP mediates the effects of adrenaline on cardiac Purkinje fibres. *Nat. New Biol.* 240:181–183.
13. Harvey, R. D., and R. E. Ten Eick. 1988. Characterization of the inward-rectifying potassium current in cat ventricular myocytes. *J. Gen. Physiol.* 91:593–615.
14. Biermans, G., J. Vereecke, and E. Carmeliet. 1987. The mechanism of the inactivation of the inward-rectifying K current during hyperpolarizing steps in guinea-pig ventricular myocytes. *Pfluegers Arch. Eur. J. Physiol.* 410:604–613.
15. Lefkowitz, R. J., J. M. Stadel, and M. G. Caron. 1983. Adenylate cyclase-coupled beta-adrenergic receptors: structure and mechanisms of activation and desensitization. *Annu. Rev. Biochem.* 52:159–186.
16. Tsien, R. W. 1974. Effects of epinephrine on the pacemaker potassium current of cardiac Purkinje fibers. *J. Gen. Physiol.* 64:293–319.
17. Egan, T. M., D. Noble, S. J. Noble, T. Powell, and V. W. Twist. 1987. An isoprenaline activated-sodium-dependent inward current in ventricular myocytes. *Nature (Lond.)*. 328:634–637.
18. Rosen, M. R., R. M. Weiss, and P. Danilo, Jr. 1984. Effect of alpha adrenergic agonists and blockers on Purkinje fiber transmembrane potentials and automaticity in the dog. *J. Pharmacol. Exp. Ther.* 231:566–571.
19. Hoffman, B. B., T. Michel, T. B. Brenneman, and R. J. Lefkowitz. 1982. Interactions of agonists with platelet α_2-adrenergic receptors. *Endocrinology*. 110:926–932.
20. Egan, T. M., D. Noble, S. J. Noble, T. Powell, V. W. Twist, and K. Yamaoka. 1988. On the mechanism of isoprenaline- and forskolin-induced depolarization of single guinea-pig ventricular myocytes. *J. Physiol. (Lond.)*. 400:299–320.
21. Bahinski, A., A. C. Nairn, P. Greengard, and D. C. Gadsby. 1989. Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes. *Nature (Lond.)*. 340:718–721.
22. Harvey, R. D., and J. R. Hume. 1989. Autonomic regulation of a chloride current in heart. *Science (Wash. DC)*. 244:983–985.
23. Arch, J. R. S., A. T. Ainsworth, M. A. Cawthorne, V. Piercy, M. V. Sennitt, V. E. Thody, C. Wilson, and S. Wilson. 1984. Atypical β-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature (Lond.)*. 309:163–165.
24. Bond, R. A., and D. E. Clarke. 1988. Agonist and antagonist characterization of a putative adrenoceptor with distinct pharmacological properties from the α- and β- subtypes. *Br. J. Pharmacol.* 95:723–734.
25. Broadley, K. J., A. G. Roach, and K. L. Williamson. 1985. Positive inotropic and chronotropic effects of noradrenaline which cannot be explained by α- or β-adrenoceptor stimulation. *Br. J. Pharmacol.* 86(Suppl.):699P.
26. Walter, M., H. Lemoine, and A. J. Kaumann. 1984. Stimulant and blocking effects of optical isomers of pindolol on the sinoatrial node and trachea of guinea pig. Role of β-adrenoceptor subtypes in the dissociation between blockade and stimulation. *Naunyn-Schmiedebergs Arch. Pharmacol.* 327:159–175.
27. Kaumann, A. J. 1989. Is there a third heart β-adrenoceptor? *TIPS (Trends Pharmacol. Sci.)* 10:316.
28. Bojanic, D., J. D. Jansen, S. R. Nahorski, and J. Zaagsma. 1985. Atypical characteristics of the β-adrenoceptor mediating cyclic AMP generation and lipolysis in the rat adipocyte. *Br. J. Pharmacol.* 84:131–137.
29. Emorine, L. J., S. Marullo, M.-M. Briend-Sutren, G. Patey, K. Tate, C. Delavier-Klutchko, and A. D. Strosberg. 1989. Molecular characterization of the human β_3-adrenergic receptor. *Science (Wash. DC)*. 245:1118–1121.
30. Armstrong, C. M. 1971. Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. *J. Gen. Physiol.* 58:413–437.
31. Strichartz, G. R. 1973. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.* 62:37–57.