Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 295–299, January 1996
Physiology

cAMP compartmentation is responsible for a local activation of cardiac Ca^{2+} channels by β-adrenergic agonists

(Ca^{2+} current/cardiac myocytes/forskolin/isoprenaline/cAMP/compartmentation)

JONAS JUREVIČIUS* AND RODOLPHE FISCHMEISTER†

Laboratoire de Cardiologie Cellulaire et Moléculaire, Institut National de la Santé et de la Recherche Médicale, CJF 92-11, Université de Paris-Sud, Faculté de Pharmacie, F-92296 Châtenay-Malabry, France

Communicated by Erwin Neher, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany, September 14, 1995 (received for review June 12, 1995)

ABSTRACT **The role of cAMP subcellular compartmentation in the progress of β-adrenergic stimulation of cardiac L-type calcium current (I_{Ca}) was investigated by using a method based on the use of whole-cell patch-clamp recording and a double capillary for extracellular microperfusion. Frog ventricular cells were sealed at both ends to two patch-clamp pipettes and positioned approximately halfway between the mouths of two capillaries that were separated by a 5-μm thin wall. I_{Ca} could be inhibited in one half or the other by omitting Ca^{2+} from one solution or the other. Exposing half of the cell to a saturating concentration of isoprenaline (ISO, 1 μM) produced a nonmaximal increase in I_{Ca} (347 ± 70%; n = 4) since a subsequent application of ISO to the other part induced an additional effect of nearly similar amplitude to reach a 673 ± 130% increase. However, half-cell exposure to forskolin (FSK, 30 μM) induced a maximal stimulation of I_{Ca} (561 ± 55%; n = 4). This effect was not the result of adenylyl cyclase activation due to FSK diffusion in the nonexposed part of the cell. To determine the distant effects of ISO and FSK on I_{Ca}, the drugs were applied in a zero-Ca solution. Adding Ca^{2+} to the drug-containing solutions allowed us to record the local effect of the drugs. Dose–response curves for the local and distant effects of ISO and FSK on I_{Ca} were used as an index of cAMP concentration changes near the sarcolemma. We found that ISO induced a 40-fold, but FSK induced only a 4-fold, higher cAMP concentration close to the Ca^{2+} channels, in the part of the cell exposed to the drugs, than it did in the rest of the cell. cAMP compartmentation was greatly reduced after inhibition of phosphodiesterase activity with 3-isobutyl-methylxanthine, suggesting the colocalization of enzymes involved in the cAMP cascade. We conclude that β-adrenergic receptors are functionally coupled to nearby Ca^{2+} channels via local elevations of cAMP.**

A number of hormones and neuromediators regulate cell function via cAMP-dependent phosphorylation. In cardiac myocytes, these include prostaglandin E_1 (1, 2), glucagon (3, 4), histamine (5), noradrenaline (1, 2, 6–8), or vasoactive intestinal peptide (9). All these agonists, while activating specific receptors at the cardiac cell membrane, produce a sequence of events that includes (*i*) activation of stimulatory guanine nucleotide binding protein G_s, (*ii*) activation of adenylyl cyclase, (*iii*) accumulation of cAMP, (*iv*) activation of cAMP-dependent protein kinase, and finally (*v*) phosphorylation of substrate proteins such as L-type Ca^{2+} channels, glycogen phosphorylase, phospholamban, troponin I, C protein, etc. Accumulation of cAMP and enhanced phosphorylation may also result from direct activation of adenylyl cyclase by forskolin (FSK) (10, 11) or from hormonal or pharmacological inhibition of cAMP phosphodiesterase activity (3, 12, 13). Yet, the effectiveness of each cAMP-elevating agent to produce a functional response (namely, a cardiac inotropic, lusitropic, or chronotropic effect) varies considerably, although doses may be equipotent at increasing cellular cAMP levels (1–9, 14). This suggests that the cardiac myocyte has the ability to discriminate among different external stimuli activating a common signaling pathway. One possible way to do so is to accumulate cAMP in different compartments, thus making the nucleotide available for phosphorylation of only a limited number of substrates. Although this hypothesis has been documented by a number of *in vitro* biochemical studies (1, 2, 15–18), it has never been directly tested in an intact single cell.

It was our aim to examine this hypothesis in a single cardiac myocyte in relation to the regulation of the L-type Ca^{2+} channel current (I_{Ca}). More specifically, we examined the role of cAMP compartmentation on I_{Ca} by using two cAMP-elevating agents: the β-adrenergic agonist isoprenaline (ISO) and the adenylyl cyclase activator FSK.

MATERIALS AND METHODS

Patch-Clamp Studies with Frog Ventricular Myocytes. Ventricular cells were enzymatically dispersed from frog (*Rana esculenta*) heart with a combination of collagenase (Yakult Pharmaceutical, Tokyo) and trypsin (Sigma) as described (19). The isolated cells were stored in standard Ringer's solution and kept at 4°C until use (2–48 hr after dissociation). Rod-shaped Ca^{2+}-tolerant frog ventricular myocytes 250–400 μm long were sealed at both ends to two patch-clamp pipettes (1.0–1.5 MΩ and a whole-cell recording condition was established for both electrodes by using two patch-lamp amplifiers (RK400, Biologic, Grenoble, France). One electrode (EL_1) was under voltage-clamp condition to measure I_{Ca}. I_{Ca} was measured by depolarizing the cell every 8 s to 0 mV for 200 ms from a holding potential of −80 mV. K^+ currents were blocked by replacing all K^+ with intracellular and extracellular Cs^+ (19), and the fast Na^+ current was blocked by tetrodotoxin. I_{Ca} was measured as the difference between peak inward current and the current at the end of the 200-ms pulse (19). The other electrode (EL_2) was under current-clamp conditions (at zero current) to allow the measurement of membrane potential at the most remote part of the cell. The voltage signal measured by EL_2 (V_2) was compared to the clamp potential (V_c) provided by the voltage stimulator. The difference $V_c - V_2$ was amplified 0–3 times and added to V_c at the voltage input of the patch-clamp amplifier connected to EL_1. Under these conditions, even when the peak I_{Ca} amplitude reached up to 3 nA,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: I_{Ca}, L-type calcium current; IBMX, 3-isobutyl-1-methylxanthine; ISO, isoprenaline; FSK, forskolin; ACh, acetylcholine; $[cAMP]_l$ and $[cAMP]_d$, local and distant [cAMP], respectively.

*Permanent address: Kaunas Medical Academy, Institute of Cardiology, Laboratory of Membrane Biophysics, Kaunas 3007, Lithuania.
†To whom reprint requests should be addressed.

the voltage at EL_2 differed from the voltage at EL_1 by <3 mV. All experiments were done at room temperature (19–26°C).

Microperfusion of Single Cardiac Myocytes. After breaking of the patch membrane at the tips of both electrodes and establishment of whole-cell recording conditions, EL_1 and EL_2 were moved separately so that the cell was positioned transversely at the mouth of two adjacent capillaries (square section 400 μm × 400 μm) made out of Plexiglas and separated by an intermediate wall ≈5 μm thick. The cell was thus exposed to two solutions (S1 and S2). Mixing of the solutions at the cell membrane was minimized by (*i*) pressing the cell against the wall, (*ii*) positioning the tips of EL_1 and EL_2 inside the mouth of the capillaries, and (*iii*) pressure ejection of the solutions out of the capillaries at a linear velocity of ≈2 cm/s. Estimation of the portion of the cell membrane exposed to each solution was achieved by briefly omitting Ca^{2+} from one solution or the other (zero-Ca solution). When both S1 and S2 were zero-Ca solutions, I_{Ca} current was fully abolished. When only S1 or S2 was a zero-Ca solution, I_{Ca} amplitude was reduced due to blockage of the channels exposed to zero Ca. In this case, the reduction of I_{Ca} was used to quantify the portion of the cell exposed to S1 and S2 (see, e.g., Fig. 1). In few experiments, the zero-Ca solution was replaced by a solution containing 1 mM $CdCl_2$ with no difference in results. We also verified that the microperfusion system was symmetrical by omitting Ca^{2+} successively from S1 (zero-Ca/Ca) and then from S2 (Ca/zero-Ca) only. The resulting currents summed to the I_{Ca} amplitude obtained when S1 and S2 contained Ca (Ca/Ca).

Solutions and Drugs. The control Ringer's solution contained 107 mM NaCl, 10 mM Hepes, 20 mM CsCl, 4 mM $NaHCO_3$, 0.8 mM NaH_2PO_4, 1.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM D-glucose, 5 mM sodium pyruvate, and 3×10^{-7} M tetrodotoxin (pH 7.4 adjusted with NaOH). The zero-Ca solution was obtained by omitting $CaCl_2$ from the above solution (no Ca buffer added). Patch electrodes were filled with control internal solution that contained 119.8 mM CsCl, 5 mM EGTA (acid form), 4 mM $MgCl_2$, 5 mM creatine phosphate (disodium salt), 3.1 mM Na_2ATP, 0.42 mM Na_2GTP, 0.062 mM $CaCl_2$ (pCa 8.5), and 10 mM Hepes (pH 7.1 adjusted with CsOH). ISO was prepared as stock solutions of 1 mM in 5 mM ascorbic acid. FSK was prepared as stock solutions of 10 mM in 96% ethanol and each solution tested contained a similar amount of ethanol (<0.3%). Tetrodotoxin was from Latoxan (Rosans, France) and all other drugs were from Sigma.

RESULTS

Partial exposure of a frog cardiac myocyte to drugs and hormones was achieved by positioning the cell transversely at the mouth of two adjacent capillaries separated by a thin wall. Two solutions superfused these two regions of the cell. I_{Ca} could be inhibited in one region or the other by omitting Ca^{2+} (no Ca buffer added) from one solution or the other. When doing so, whole-cell I_{Ca} amplitude was reduced by 44–57%. In each experiment, this reduction in I_{Ca} was used to quantify the portion of the cell membrane exposed to each solution (Fig. 1 *A* and *B*). Exposing half of the cell to a saturating concentration of ISO (1 μM; Fig. 1*A*) or FSK (30 μM, Fig. 1*B*) induced a clear increase in I_{Ca} (8, 11). In these initial experiments, both drugs were used at concentrations about 50-fold larger than the EC_{50} values for I_{Ca} stimulation (see, e.g., Fig. 3). However, the effects of ISO and FSK differed markedly. Half-cell exposure to FSK induced a maximal stimulation of I_{Ca} (561 ± 55% over control amplitude; mean ± SEM, n = 4) since application of FSK to the other part produced little additional effect (total increase, 601 ± 51%; Fig. 1*B*). This shows that the Ca^{2+} channels in the portion of the cell not exposed to FSK were already fully activated by the presence of the drug in the other part. However, half-cell exposure to ISO produced a faster but nonmaximal increase in I_{Ca} (347 ± 71%, n = 4) since a subsequent application of ISO to the other part induced an additional effect of nearly similar amplitude (total increase, 673 ± 130%; Fig. 1*A*). This demonstrates that the portion of the cell not exposed to ISO was little affected by the presence of ISO in the other part. In other words, FSK induced a larger distant effect than ISO.

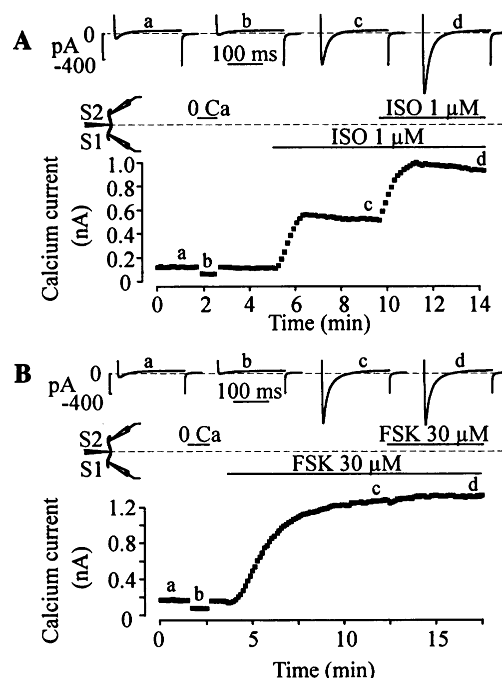

FIG. 1. Time course of the effects of ISO (*A*) and FSK (*B*) on I_{Ca} in frog ventricular cells. As indicated by the drawings, the cell was always positioned approximately halfway between S1 and S2. The upper current traces were obtained at the times indicated by the corresponding letters in the lower graphs. In *A* and *B*, S1 and S2 initially contained control Ringer's solution. During a short period, Ca^{2+} were omitted from S2 (zero Ca) and I_{Ca} rapidly decreased by 44% in *A* and 52% in *B*. If there is a homogeneous distribution of Ca^{2+} channels on the cell membrane, then 44% in *A* and 52% in *B* of the total cell surface was exposed to S2, the rest of the cell being exposed to S1. After reintroduction of Ca^{2+} in S2, ISO (1 μM, in *A*) or FSK (30 μM, in *B*) were added to S1 or to both S1 and S2 during the periods indicated.

A trivial explanation of this difference is that FSK diffuses in the remote part of the cell and activates adenylyl cyclase even in the portion of the cell not directly exposed to the drug. To check this hypothesis, we examined the effects of acetylcholine (ACh), a muscarinic receptor agonist that antagonizes the effects of ISO (19) or FSK (11) on I_{Ca} through inhibition of adenylyl cyclase (7, 19). ACh (1–3 μM) strongly antagonizd the effects of ISO (10 μM) or FSK (30 μM) on I_{Ca} when applied to the portion of the cell exposed to these drugs. However, ACh had no effect (at up to 10 μM) when applied to the other side (data not shown). Thus, contamination between adjacent solutions was not significant and the distant effects of ISO or FSK were not due to activation of adenylyl cyclase in the region not exposed to the drugs. Therefore, the distant effects of ISO and FSK most likely reflect the intracellular diffusion of cAMP into the cytoplasm underneath the membrane in that region.

The overall effect of ISO or FSK on I_{Ca} is a combination of a local effect (i.e., the stimulation of the Ca^{2+} channels in the part of the cell exposed to the agonist) and a distant effect (i.e., the stimulation of the Ca^{2+} channels in the remote part of the cell not exposed to the agonist). It was possible, though, to separate the two effects by applying ISO or FSK in a Ca^{2+}-free solution (Fig. 2). In this case, an increase in I_{Ca} reflects only

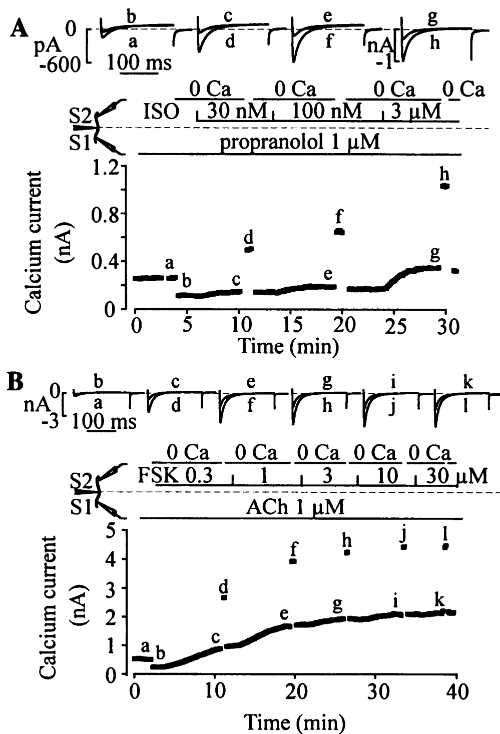

FIG. 2. Local vs. distant effects of ISO and FSK on I_{Ca}. (*A* and *B*) Time course of the changes of I_{Ca} in frog ventricular cells. As indicated by the drawings, the cell was always positioned approximately halfway between S1 and S2. The upper current traces were obtained at the times indicated by the corresponding letters in the lower graphs. S1 contained, respectively, 1 μM propranolol (*A*) or 1 μM ACh (*B*) throughout the entire experiment to eliminate any effect of contamination of S1 by ISO or FSK. After 3–4 min, Ca^{2+} was omitted from S2 (zero Ca) and I_{Ca} decreased by 55% in *A* and 53% in *B*. Increasing concentrations of ISO (*A*) or FSK (*B*) were successively added to S2 (in zero Ca) and produced a distant effect on I_{Ca}. When a steady state was achieved, Ca^{2+} was briefly reintroduced in S2, with ISO (*A*) or FSK (*B*), to evaluate the local effect of the drugs on I_{Ca}.

the distant effect of the drugs since the only Ca^{2+} channels generating a current are those not exposed to the drugs. Adding Ca^{2+} in the drug-containing solutions for short periods elicits an additional increase in I_{Ca} that now reflects the local effect of the drugs. Two such experiments where distant and local effects of ISO (Fig. 2*A*) and FSK (Fig. 2*B*) were measured simultaneously with increasing concentrations of agonists are presented. To fully eliminate residual contamination between solutions, propranolol (1 μM, Fig. 2*A*), a β-adrenergic antagonist, or ACh (1 μM, Fig. 2*B*) was added to the distant part. Major differences were found between the local and distant effects of ISO and FSK. The dose–response curves for the effects of FSK show that distant and local effects differ only by a $\approx$3-fold reduction in the sensitivity to the agonist (Fig. 3*B*). However, the differences between local and distant effects of ISO are much more pronounced (Fig. 3*A*). It appears that at a concentration producing an equivalent local increase in I_{Ca}, ISO induced a much smaller distant effect than FSK.

This laboratory has demonstrated (8) that β-adrenergic regulation of I_{Ca} is entirely due to cAMP-dependent phosphorylation. Consequently, the large difference between the local and distant effects of ISO may arise (*i*) from differences between local and distant cAMP concentrations, e.g., due to a limited diffusion of cAMP within the cell and/or (*ii*) from a higher sensitivity of local Ca^{2+} channels to cAMP-dependent phosphorylation. The latter could be the result of an interaction between the G protein G_s and Ca^{2+} channels (20), which

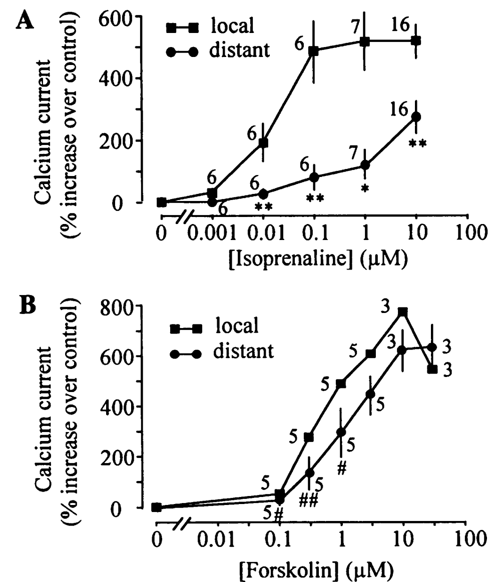

FIG. 3. Concentration–response curves of ISO (*A*) and FSK (*B*) on I_{Ca}. Local (■) and distant (●) effects of the drugs were obtained in several experiments performed as in Fig. 2 *A* and *B*. ISO or FSK were applied only to S2, which superfused 43–57% of the total cell surface. S1 contained either 1 μM propranolol (*A*) or 1 μM ACh (*B*). The points show the mean ± SEM of the number of cells indicated near the symbols. Statistically significant differences between data points are indicated at the 0.05 (#), 0.005 (##), 0.001 (*), and 0.0005 (**) levels (*t* test).

has been shown to prime the channels for cAMP-dependent phosphorylation (21). To distinguish between these two hypotheses, local and distant effects of ISO were reevaluated in the presence of 3-isobutyl-1-methylxanthine (IBMX), a nonselective inhibitor of phosphodiesterase activity. Fig. 4*A* shows an experiment in which the distant and local effects of three increasing concentrations of ISO (10 nM, 100 nM, and 1 μM) were evaluated first in the similar conditions as in Fig. 2*A* and, subsequently, in the presence of 100 μM IBMX added to ISO. As shown (13), IBMX alone had no effect on I_{Ca}. However, when 100 μM IBMX was added to the part of the cell exposed to ISO, it strongly potentiated the distant effect of ISO on I_{Ca} (Fig. 4*A*). On average, IBMX significantly increased (by a factor of 3–4) the distant effect of all three concentrations of ISO on I_{Ca} (Fig. 4*B*). By comparison, IBMX had a much smaller influence on the local effect of ISO. In four other cells, the effect of ISO was examined in the presence of 30 μM IBMX on both parts of the cell. In these cells, the distant effect of ISO (0.3 μM) became identical to its local effect (data not shown). Thus, these data suggest that a limited diffusion of cAMP rather than an additional stimulatory effect of β-adrenergic agonists on I_{Ca} appears to be responsible for the large differences between local and distant effects of ISO.

The simultaneous determination of local and distant effects of ISO and FSK on I_{Ca} allows an estimate of the degree of cAMP compartmentation achieved by each agonist. Indeed, I_{Ca} variations can be used as an index of cAMP concentration changes near the Ca^{2+} channels (8, 13). When exogenous cAMP is perfused inside a frog ventricular myocyte, a relationship is obtained between I_{Ca} and cAMP concentration, which is well fitted by the Michaelis equation (22). We thus assumed that local (E_l) and distant (E_d) effects of ISO and FSK on I_{Ca} reflect the changes in local ($[cAMP]_l$) and distant ($[cAMP]_d$) cAMP concentrations, respectively. When normalized to the maximal increase in I_{Ca}, E_l and E_d were described by the equations: $E_l = [cAMP]_l/([cAMP]_l + K_d)$ and $E_d = [cAMP]_d/([cAMP]_d + K_d)$. The dissociation constant K_d reflects an "apparent affinity" of Ca^{2+} channels for cAMP.

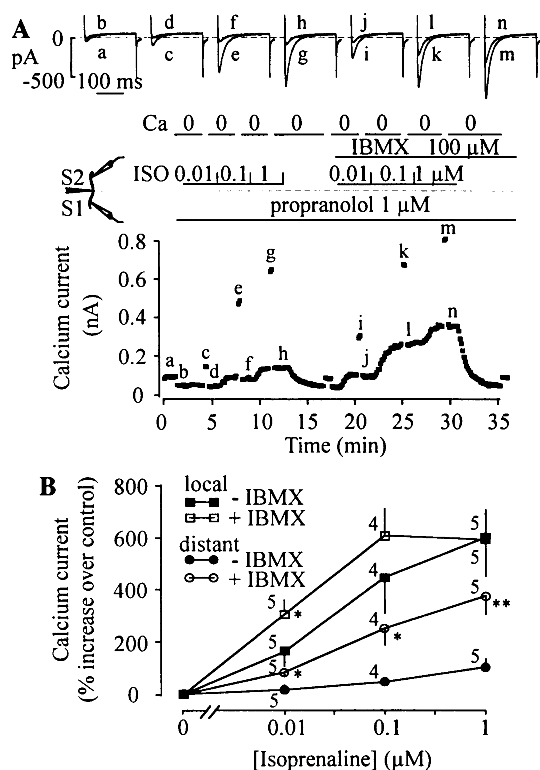

FIG. 4. Role of phosphodiesterase activity in the effect of ISO on I_{Ca}. (*A*) Time course of the changes of I_{Ca} in a frog ventricular cell. As indicated by the drawing, the cell was positioned approximately halfway between S1 and S2. S1 contained 1 μM propranolol throughout the entire experiment. After 2 min, Ca^{2+} was omitted from S2 (zero Ca) and I_{Ca} decreased by 45.6%. Increasing concentrations of ISO (10 nM, 100 nM, and 1 μM) were successively added to S2 (in zero Ca) during the periods indicated and produced a small distant effect on I_{Ca}. When a steady state was achieved, Ca^{2+} was briefly reintroduced in S2 with ISO to evaluate the local effect of the drug on I_{Ca}. At the end of this protocol, ISO was washed out and the current returned to control level. The same protocol was then repeated in the presence of IBMX (100 μM) added to S2. The upper current traces were obtained at the times indicated by the corresponding letters in the lower graph. (*B*) Concentration–response curves of ISO on I_{Ca}. Local (■ and □) and distant (● and ○) effects of ISO were obtained as in *A* in the absence (■ and ●) and presence (□ and ○) of 100 μM IBMX. ISO with or without IBMX was applied only to S2, which superfused 45.5 ± 3.3% (mean ± SEM, n = 5) of the total cell surface. S1 contained 1 μM propranolol. The points show the mean ± SEM of the number of cells indicated near the symbols. Statistically significant differences between data points in IBMX vs. control are indicated at the 0.05 (*) and 0.01 (**) levels (t test).

However, this parameter disappears when introducing the coefficient $\alpha = [cAMP]_l/[cAMP]_d$, which represents the gradient of local vs. distant cAMP concentration. We thus obtain the equation:

$$E_d = E_l/[\alpha + (1 - \alpha)E_l]. \quad [1]$$

This equation was used to fit the experimental data from Fig. 3 for the effect of ISO and FSK on I_{Ca}. The data are plotted as E_d vs. E_l in Fig. 5. A fit of the data to Eq. 1 leads to α values of 40.3 and 3.7 for ISO and FSK, respectively (Fig. 5). Thus, during ISO application, cAMP concentration was found to be 40-fold higher in the part of the cell exposed to the β-agonist than in the rest of the cell. However, during FSK application, local and distant levels of cAMP differed only by a factor of ≈4 (Fig. 5). Therefore, ISO induced a 10-fold larger cAMP compartmentation than FSK.

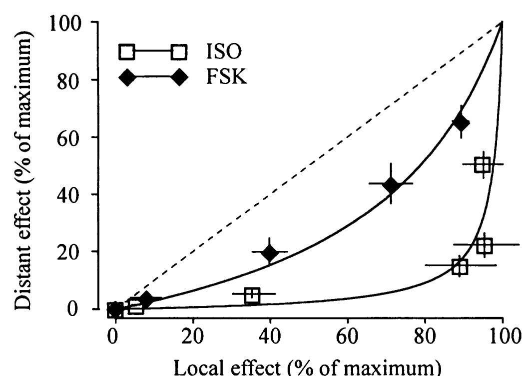

FIG. 5. Relationship between distant and local effects of ISO or FSK on I_{Ca}. The individual points were generated from the mean values ± SEM in Fig. 3*A* (for ISO, □) and Fig. 3*B* (for FSK, ◆) normalized to the maximal increase in I_{Ca}. The data points were fitted to the equation: $y = x/[\alpha + (1 - \alpha)x]$ (for further details, see text). Values derived for α were 40.3 for ISO and 3.7 for FSK, which is representative of the respective gradients between local and distant cAMP concentrations. The dotted line indicates a 1:1 relationship between the parameters.

DISCUSSION

Hormonal regulation of ion channel activity often involves a sequence of biochemical events, such as the production or degradation of a second messenger and the activation of a protein kinase. A prototype of this type of regulation is the β-adrenergic regulation of cardiac L-type Ca^{2+} channels (7). However, little is known about the subcellular localization of the biochemical events. It is reasonable to assume that a hormone should be more potent if its action is mainly local (i.e., if all the sequential events take place in a restricted region near the channel) than if one (or several) intermediate reactant(s) are allowed to diffuse away and affect remote channels in a distant manner, as well as other processes within the cell. Thus, it was our aim to address these questions and their relevance in the case of cardiac Ca^{2+} channel regulation. By using a microperfusion technique combined with the whole-cell patch-clamp technique, we have demonstrated in frog ventricular myocytes that the local application of a β-adrenergic agonist preferentially stimulates L-type Ca^{2+} channels near the locus of activated receptors. In contrast, a local activation of adenylyl cyclase by FSK results in a spatially generalized enhancement of Ca^{2+} channel activity. In addition, local inhibition of cAMP phosphodiesterase activity strongly reduced the nonuniformity in β-adrenergic response, suggesting a colocalization of the enzymes involved in the cAMP cascade. Thus, our results suggest that β-adrenergic receptors are functionally coupled to nearby Ca^{2+} channels via local elevations of cAMP.

A number of biochemical *in vitro* studies have examined the degree of accumulation of cAMP or activation of cAMP-dependent phosphorylation in particulate and soluble fractions of cardiac myocytes challenged with β-adrenergic agonists (1, 2, 15–18), prostaglandin E_1 (1, 2, 15), FSK (15, 18), or phosphodiesterase inhibitors (7, 18). ISO is generally found to produce a larger accumulation of cAMP in the particulate relative to soluble fraction while FSK or phosphodiesterase inhibitors affect both compartments in a more equal fashion (16, 18). In an elegant study on canine ventricular myocytes, Hohl and Li (18) demonstrate that about 45% of the total cAMP is found in the particulate fraction in response to 1–100 nM ISO but that this fraction declined to <20% when 10 μM IBMX was added to ISO, although total cAMP still increased ≈3-fold (18). This suggests that phosphodiesterase activity may be responsible for a limited elevation of cAMP in the soluble fraction in response to ISO. Our results with IBMX are

in full agreement with this hypothesis. Indeed, the data in Fig. 4 show that IBMX reduces the difference between the local and distant effects of ISO on I_{Ca}. Thus, both the biochemical and functional data support the hypothesis that, during β-adrenergic stimulation, cAMP phosphodiesterases play a critical role in limiting the amount of cAMP diffusing from membrane to cytosol. This may be of major relevance for the cell function since particulate, rather than soluble, cAMP and cAMP-dependent protein kinase appear to play a dominant role in the phosphorylation of membrane and contractile proteins and in the regulation of the inotropic state of the heart (15–18).

Because FSK is a universal activator of adenylyl cyclase (10), it is reasonable to assume that the number of cyclases activated by FSK is larger than that coupled to β-adrenergic receptors. In spite of this, the effect of FSK on I_{Ca} develops with a 2- to 3-fold slower time scale than the effect of β-adrenergic agonists at doses that are equipotent in their effects on I_{Ca} (20, 23). This difference has been attributed to some concomitant inhibitory effects of FSK (23, 24) or to the presence of an additional activatory mechanism in β-adrenergic stimulation such as a priming effect of the G protein G_s on cAMP-dependent Ca^{2+} channel phosphorylation (21). However, based on the present study, we speculate that these differences in time course may rather be due to differences in the degree of cAMP accumulation near the Ca^{2+} channels. Indeed, our data suggest that β-adrenergic receptors are more efficiently coupled to adenylyl cyclases than FSK and that these cyclases are colocalized with Ca^{2+} channels. Consequently, β-adrenergic receptor activation will result in a rapid but local increase in cAMP concentration while FSK activation will result in a slower but more homogenous increase in cAMP level. Thus, cAMP compartmentation in the vicinity of Ca^{2+} channels may provide a "delimited" pathway for β-adrenergic regulation of cardiac calcium current.

The functional difference between ISO and FSK effects on intracellular cAMP distribution suggests a number of experiments: (*i*) the characterization of the phosphodiesterase isoforms involved in the cAMP compartmentation; (*ii*) the determination of the mechanisms coupling β-adrenergic receptors and phosphodiesterase activity (cAMP elevation, G proteins, etc.?); (*iii*) the respective contribution of β1- and β2-adrenergic receptors subtypes in these mechanisms; (*iv*) the functional role of cAMP compartmentation in mammalian cardiac myocytes; (*v*) the potential alteration in the cell capacity to compartmentalize cAMP in diseased hearts, particularly during heart failure, etc.; (*vi*) the role of other members of the β-adrenergic signaling cascade (such as the β-adrenergic receptor kinase, cAMP-dependent protein kinase subunits, and phosphatases) in the functional coupling between receptors and Ca^{2+} channels; and (*vii*) extension of these studies to other hormones and neuromediators that activate the same cAMP pathway but yet produce different cardiac inotropic, chronotropic, or lusitropic effects. Indeed, our observation that, in an intact cell, two different signals activating the cAMP cascade produce different intracellular cAMP distributions suggests that cAMP compartmentation may provide a general means by which a cell discriminates among different external stimuli acting via a common signaling pathway.

We thank P. Lechêne, F. Lefèbvre, I. Ribaud, and A. Navalinskas for technical help, and Drs. J.-L. Mazet, P.-F. Méry, L. Hove-Madsen, R. Ventura-Clapier, V. Veksler, J. Hoerter, O. Blondel, and L. Marraffa for helpful discussions. J.J. was a recipient of a fellowship from the Institut National de la Santé et de la Recherche Médicale and of an Invited Professorship from the Faculty of Pharmacy, Châtenay-Malabry, France.

1. Brunton, L. L., Hayes, J. S. & Mayer, S. E. (1979) *Nature (London)* **280,** 78–80.
2. Hayes, J. S., Brunton, L. L. & Mayer, S. E. (1980) *J. Biol. Chem.* **255,** 5113–5119.
3. Méry, P.-F., Brechler, V., Pavoine, C., Pecker, F. & Fischmeister, R. (1990) *Nature (London)* **345,** 158–161.
4. Wolf, A. A. & Levi, R. (1986) *Circ. Res.* **58,** 1–16.
5. Levi, R. C. & Alloatti, G. (1988) *J. Pharmacol. Exp. Ther.* **246,** 377–383.
6. Brodde, O. E. (1991) *Pharmacol. Rev.* **43,** 203–242.
7. Hartzell, H. C. (1988) *Prog. Biophys. Mol. Biol.* **52,** 165–247.
8. Hartzell, H. C., Méry, P.-F., Fischmeister, R. & Szabo, G. (1991) *Nature (London)* **351,** 573–576.
9. Xenopoulos, N. P. & Applegate, R. J. (1994) *Am. J. Physiol.* **266,** H399–H405.
10. Seamon, K. B. & Daly, J. W. (1986) *Adv. Cyclic Nucleotides Protein Phosphoryl. Res.* **20,** 1–150.
11. Hartzell, H. C. & Fischmeister, R. (1987) *Mol. Pharmacol.* **32,** 639–645.
12. Beavo, J. A. (1988) *Adv. Second Messengers Phosphoryl. Res.* **22,** 1–38.
13. Fischmeister, R. & Hartzell, H. C. (1990) *Mol. Pharmacol.* **38,** 426–433.
14. Kenakin, T. P., Ambrose, J. R. & Irving, P. E. (1991) *J. Pharmacol. Exp. Ther.* **257,** 1189–1197.
15. Buxton, I. L. O. & Brunton, L. L. (1983) *J. Biol. Chem.* **258,** 10233–10239.
16. Aass, H., Skomedal, T. & Osnes, J.-B. (1988) *J. Mol. Cell. Cardiol.* **20,** 847–860.
17. Rapundalo, S. T., Solaro, R. J. & Kranias, E. G. (1989) *Circ. Res.* **64,** 104–111.
18. Hohl, C. M. & Li, Q. (1991) *Circ. Res.* **69,** 1369–1379.
19. Fischmeister, R. & Hartzell, H. C. (1986) *J. Physiol. (London)* **376,** 183–202.
20. Yatani, A. & Brown, A. M. (1991) *Science* **245,** 71–74.
21. Cavalié, A., Allen, T. J. A. & Trautwein, W. (1991) *Pflügers Arch.* **419,** 433–443.
22. Fischmeister, R. & Hartzell, H. C. (1987) *J. Physiol. (London)* **387,** 453–472.
23. Frace, A. M., Méry, P.-F., Fischmeister, R. & Hartzell, H. C. (1993) *J. Gen. Physiol.* **101,** 337–353.
24. Boutjdir, M., Méry, P.-F., Hanf, R., Shrier, A. & Fischmeister, R. (1991) *Mol. Pharmacol.* **38,** 758–765.