Published in final edited form as:

J Basic Clin Physiol Pharmacol. 1996 ; 7(4): 347–362.

EFFECTS OF THEOPHYLLINE AND DIBUTYRYL-cAMP ON ADENOSINE RECEPTORS AND HEART RATE IN CULTURED CARDIOCYTES

Dalia El-Ani¹, Kenneth A. Jacobson², and Asher Shainberg^{1,*}

¹The Otto Meyer hoff Drug Receptor Center, Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

²Section of Molecular Recognition, Laboratory of Bioorganic Chemistry, NIH, NIDDK, Bethesda, Maryland 20892, USA

Abstract

The effects of chronic exposure to the adenosine antagonist theophylline (Theo) and dibutyryl cyclic-AMP, a membrane-permeant derivative of the second messenger 3',5'-cyclic-AMP (cAMP), on contractions and adenosine receptor levels in cultured cardiocytes were studied. Binding of the A₁-adenosine receptor antagonist [³H]8-cyclopentyl-1,3-dipropylxanthine ([³H]CPX) was used to monitor the level of the receptors in intact cardiocytes. Both Theo and cAMP stimulated the rate of contraction and also increased the density of adenosine receptors. The B_{max} value for [³H]CPX binding to intact cardiocytes was increased by 45–47% following 4 days of exposure to either 50 μM Theo or 100 μM cAMP. Scatchard analysis indicated that the affinity of the A₁ receptors for [³H]CPX remained unchanged (K_d 0.1–0.2 nM). No significant differences were observed in protein content or in cell number. A linear correlation was achieved between the level of A₁-adenosine receptors and heart rate at various Theo and dibutyryl-cAMP concentrations, although Theo was more efficient in elevation of the receptor density. Increases of 82, 78, 138 and 235% in A₁ receptor density and increases of 63, 59, 66 and 150% in heart rate were obtained following 5 days of treatment with 1, 10, and 1000 μM of Theo, respectively. It is concluded that there is a linkage between the rate of cardiac contractions and the level of adenosine receptors. Thus, changes in the density of adenosine receptors may compensate for chronic drug-induced changes in cardiac contractile activity so as to restore conditions to the normal state.

Keywords

CPX; heart rate; theophylline; cAMP; adenosine receptors

INTRODUCTION

Adenosine acting at extracellular receptors modulates important physiological functions in the heart. The responses to adenosine include negative chronotropic, dromotropic and

* Author for correspondence.

inotropic effects via A₁ receptors, or vasodilatation via A₂ receptors /1–5/. In conditions of stress, such as hypoxia or ischemia, adenosine levels in the extracellular fluid of the heart rise dramatically. Activation of A₁-adenosine receptors is coupled to inhibition of adenylyl cyclase through a guanine nucleotide binding protein /6–13/, opening of K⁺ channels /14–16/, and inhibition of Ca²⁺ channels /17/. Because of these effects on cardiac function, adenosine analogs (agonists) can potentially be used as therapeutic agents /18/. Adenosine itself is widely used in the treatment of supraventricular tachycardia. Adenosine antagonists, such as theophylline, are commonly used for treating asthma and recently for treating myocardial ischemia /19,20/. The therapeutic effect of theophylline and other methylxanthines is partially mediated through antagonism of endogenous adenosine at its receptors.

Chronic exposure to theophylline or other methylxanthines has been shown to induce up-regulation of adenosine receptors in adipose tissue /21/ and brain /22–24/. A significant increase in the density of A₁-adenosine receptors was observed in hearts of theophylline-fed animals /17,25/ and in membranes isolated from chick embryo heart cultures following theophylline treatment /26/. Since Theo also inhibits the activity of phosphodiesterases and could cause elevation of intracellular cAMP by either mechanism, we studied the effects of both Theo and cAMP on heart rate and adenosine receptor levels. Recently, we have shown that drug treatment which affected heart contractility in cardiocyte cultures regulated the density of A₁-adenosine receptors. In general, agents that accelerate heart rate, such as catecholamines or thyroid hormones, caused up-regulation of the receptors, whereas carbamylcholine, which attenuates the rate of contraction, reduced the density of A₁-adenosine receptors /27/. In the present study we have demonstrated that exposure of intact rat cardiocytes to theophylline or to cAMP, both of which accelerate heart rate, leads to up-regulation of cardiac A₁-adenosine receptors. Thus, present data further indicate that contractile activity regulates the density of A₁-adenosine receptors in the heart.

MATERIALS AND METHODS

Preparation of heart cell cultures

Rat hearts (1–2 days old) were removed under sterile conditions and washed 3 times in phosphate buffered saline (PBS) to remove excess blood cells. The hearts were minced to small fragments and then gently agitated in a solution of proteolytic enzyme-RDB (IIBR, Ness-Ziona, Israel) prepared from a fig tree extract. The RDB was diluted 1:50 in PBS, at 25°C for a few cycles of 10 min each, as described previously /27–30/. The supernatant suspensions containing dissociated cells, to which medium containing 10% horse serum (Biolab, Jerusalem, Israel) was added, were centrifuged at 150 *g* for 5 min. After centrifugation, the supernatant phase was discarded and cells were resuspended in high glucose (5 mg/ml) DMEM (Biological Industries, Kibbutz Beith-Haemek, Israel) supplemented with 10% heat-inactivated horse serum and 2% chick embryo extract. The suspension of cells was diluted to 1×10⁶ cells/ml and 1.5 ml were placed in 35 mm collagen-gelatin-coated plastic culture dishes. Cultures were incubated in a humidified atmosphere of 5% CO₂, 95% air at 37°C. Confluent monolayers, which exhibit spontaneous contractions, developed in culture within 2 days. The growth medium was replaced every 3–4 days.

Radioligand binding studies

Radioligand binding studies were carried out as described previously /27/. Washing of the intact cultures after Theo treatments was necessary to removed the antagonist, especially at high doses of Theo. Therefore, before the radioligand binding assay was done the cultures were washed six times, with an interval of 3–4 minutes between each washing. Intact cells were then incubated at room temperature (23–25°C) for 60 min, with various concentrations of [³H]CPX, 1 µg/ml dipyridamole, 2 U/ml adenosine deaminase in PBS, pH 7.4. Incubation was stopped by rapidly rinsing the cells five times with cold (4–8°C) PBS. The cells were solubilized with 0.2 ml 0.5 N NaOH and neutralized upon addition of 0.1 ml 2 N Tris-HCl, pH 3.7. After addition of 3 ml of scintillation liquid radioactivity was determined using a β-counter. Non-specific binding of [³H]CPX was defined as the amount of radioactivity remaining after incubation with theophylline (5 mM). Specific [³H]CPX binding was calculated as the total radioactivity bound minus the non-specific binding (less than 20%).

Protein content and creatine kinase activity

Protein determination was performed according to Bradford's method /31/, using bovine serum albumin as a standard.

The cardiocytes were washed with cold PBS, and the cells were homogenized in the same buffer. Creatine kinase activity was determined using a CK kit (Sigma, St. Louis, MO, USA), and the product of the enzymatic activity was measured spectrophotometrically at a wavelength of 340 nm as previously described /27/.

Measurement of contractility

Changes in the contractile state of individual cells in the cardiocyte monolayers were monitored using an optical video system. A 35 mm culture dish containing growth medium was placed in a chamber containing humidified atmosphere of 5% CO₂, 95% air at 37°C. The chamber was placed on the stage of an inverted phase contrast microscope (Olympus). The cells were magnified with a 20× objective, and the image was captured by a television camera connected to a video motion detector that monitored cell movements. The analog voltage output from the motion detector was recorded (Graphtec Thermal Arraycorder WR 7500, Tokyo, Japan). For calculation of heart rate at least five cells from different culture-dishes were monitored.

Reproducibility of data

Each experiment was carried out at least in triplicate on sister cultures. Results are presented as means ± S.D. The significance of differences between the various treatments were analyzed using Student's t-test. P < 0.05 was considered significant.

Materials

Bradford solution for protein assay was purchased from Bio-Rad Lab (Munich, Germany). All other chemicals used were from Sigma Chemical Co. (St. Louis, MO, USA). [³H]CPX (sp. act. 108.3 Ci/mmol) was purchased from Dupont (Boston, MA, USA).

RESULTS

Effect of Theo on [³H]CPX binding

Since Theo stimulates the contraction of cardiocytes, we investigated whether this increase in the rate of heart contraction is associated with a change in the level of adenosine receptors. As shown in Fig. 1, [³H]CPX binding was significantly increased ($P<0.05$) in intact cardiocytes following 84 hours of 50 μ M Theo treatment. An increase of 37% in the level of A₁-adenosine receptors was achieved following this treatment (Fig. 1). Scatchard analysis shows that the affinity of the A₁ receptor for this antagonist remained nearly unchanged (Fig. 1B). Following this treatment there were no significant differences in protein content (1.13 ± 0.11 mg/dish in control and 1.05 ± 0.14 mg/dish in Theo) or in cell number. There were also no significant differences in protein content, cell number and creatine kinase activity between control and Theo (100 μ M) following longer (5 day) treatment (Table 1). However, following 5 days Theo exposure the rate of contraction and B_{max} for [³H]CPX binding increased by 57% and 138%, respectively (Table 1).

Coupling of adenosine receptor level to heart rate

Four day-old cardiocytes were treated with various concentrations of Theo for 5 days then heart rate (beats/min) and [³H]CPX binding were measured. A linear correlation was found between the level of A₁-adenosine receptors and the heart rate at various Theo concentrations (Fig. 2). Increases of 235, 138, 78 and 82% in A₁-adenosine receptor levels against increases of 130, 66, 59, 63% in heart rate were obtained following treatment with 1000, 100, 10 and 1 μ M of Theo, respectively (Fig. 2). There were no significant changes (78–85%) in [³H]CPX binding, myocyte contractility and heart rate (54–60%) below 10 μ M of Theo, although both parameters were elevated (Figs. 2 and 3).

Effects of exposure to a membrane-permeant cAMP derivative

Since Theo may cause an elevation of intracellular cAMP, the effects of treatment with this second messenger on adenosine receptor levels and on myocyte contractility were investigated. Direct application of dibutyryl-cAMP (cAMPdB) induced a significant increase in heart rate. Therefore, cardiocytes exposed chronically to this nucleotide were analyzed for heart rate and parameters of [³H]CPX binding. Four days of treatment with 100 μ M cAMPdB caused an elevation of A₁-adenosine receptor density from 24.4 to 36.0 fmol/dish without a significant change in the affinity of the receptors for the radioligand (Fig. 4). No significant difference in protein content, creatine kinase activity or in cell number was observed following this treatment; however, myocyte contractility was increased by 125% ($p<0.01$, Table 2). It was found that the rate of contraction increased by 90–130%, while the levels of A₁-adenosine receptors increased by 28% and 47% following 3 and 4 days of treatment, respectively, with dibutyryl cAMP at a concentration of 100 μ M (Fig. 5).

DISCUSSION

In this study we investigated the dynamic properties of A₁-adenosine receptors in intact rat cardiomyocytes in culture, following sustained treatment with either the adenosine antagonist Theo or the second messenger cAMP. The main finding is that there is a direct

linear correlation between the cardiac contractile rate and the density of A₁-adenosine receptor antagonist binding sites ($R=0.941$). We showed an increase of 47–235% in [³H]CPX binding ($B_{\max}$) with no significant change in the affinity of the radioligand at the receptors (K_d) upon exposure of the cardiocytes to Theo for 3–5 days (Figs. 1–2). Furthermore, this treatment caused no significant changes in CK activity, protein content and cell number (Table 1). These findings are in agreement with results obtained by others, in which Theo was administrated orally to guinea-pigs /1/ or rats /17/. In those studies the A₁ receptors were assayed using radioligand binding in cardiocyte membrane preparations rather than in intact cells, as in our study. As in the present study up-regulation of the A₁-adenosine receptor density without any significant change in affinity was reported /1,17/. Previous studies have demonstrated that A₁-adenosine receptors in fat, brain and other tissues may be up- or down-regulated following chronic drug treatment. Adenosine receptor density increases with chronic blockade by antagonists /21,24/, and decreases with chronic stimulation of the receptors by agonists /32–35/. We have shown in a previous study /27/ that norepinephrine, isoproterenol and thyroid hormones, which accelerate heart contraction rate, caused elevations in the receptor levels, whereas carbamylcholine, which attenuates the rate of contractions, reduced the level of adenosine receptors. In the present study chronic exposure to the antagonist Theo, which also stimulates the contractions of the cardiocytes, caused up-regulation of the A₁ receptors which was also accompanied by an increased rate of contraction in the cultures (Fig. 3). Similar results were obtained by Liang and Donovan /26/, who measured 33–51% increases in the receptor density as demonstrated in competition experiments of [³H]CPX with R-PIA. They also found an increase in R-PIA-elicited inhibition of adenylyl cyclase activity and reduced contractility in Theo-treated chick cardiocytes. In those experiments, A₁-adenosine receptor density was up-regulated from 20 to 33 fmol/mg protein, similar to our results. Similar results were also obtained in membranes of cultured chick atrial cardiomyocytes treated with 30 μ M 8-(-sulfophenyl)-theophylline (8-PST) for 24 hours /36/ Wu *et al.* /25/ found a larger increase in $B_{\max}$ (67%) for A₁ receptors in ventricular guinea-pig membranes. Lee *et al.* /17/ measured increases in A₁ receptor density of 22% in atrial rat membranes and 24% in ventricular rat membranes following Theo treatment. However, Shryock *et al.* /37/ found that administration of 2 μ M Theo to 15 day-old chick embryos for 44 hours did not change significantly either the number or affinity of cardiac A₁-adenosine receptors in membrane preparations. Similar results were obtained following treatment with the more selective A₁ antagonist CPX (0.5 μ M). These differences in response to Theo could be due to species differences or to the different concentrations of Theo used: 87 μ M by Lee *et al.* /17/ and 130 μ M by Wu *et al.* /25/. Nevertheless, in these two studies /17,25/ the time of exposure of the animals to the drug was equivalent, *i.e.* 2 weeks.

We have shown previously that increases in A₁ receptor levels following drug treatment are time-dependent /27/. Also, in our present experiments the influence of Theo and cAMP was time-dependent. For example, the significant increase in A₁ receptors following an exposure to Theo or cAMP for 3 or 4 days was not seen after 2 days. The difference between our results and those of Liang and Donovan /26/ (increase of 33–51%) may be related to species differences (chick atrial cardiomyocytes), and to the duration of exposure to the drug, or to the binding technique, *i.e.* either in intact cells (this study) or in membrane preparations /26/.

Moreover, washing of the cells after Theo treatment might influence the levels of ligand binding. It was reported that Theo induced adenosine production in canine myocardium /38/. Therefore, an intensive washing of the cells is needed before the radioligand binding assay. Although we replaced the culture medium daily during the Theo treatment, it is possible that the degree of up-regulation that was achieved in this study was hampered by the adenosine released from the cells.

It is possible that the changes observed in the levels of A₁ receptors are related to changes in concentration of cAMP and not to cardiac contractions. Indeed direct application of cAMP caused an increase of A₁ receptors (Figs. 4 and 5). However, an increase in adenosine receptors was obtained with Theo at 100 µM or below. At these concentrations Theo exerts minimal inhibitory effects on phosphodiesterase, less than 5% /39,40/. Hence, the increased heart rate and [³H]CPX binding in 0.1–10 µM Theo (Fig. 2) is probably not a result of elevation in intracellular cAMP through phosphodiesterase inhibition. Furthermore, when the cardiocytes were treated with T₃ for 48 h, the basal level of cAMP was only 10% higher than the control, an insignificant difference /29/, whereas the level of adenosine receptors was elevated by 56% /41/. These results support our proposal that contractile activity plays a major role in regulation of adenosine receptor level, although the possibility that cAMP influences this process is not excluded.

The elevation of A₁-adenosine receptors in rat cultures after chronic Theo treatment might be reflected by increased sensitivity to adenosine linked to functional responses, that included negative chronotropic, dromotropic and inotropic effects on the heart /1/. Thus, up-regulation of the adenosine receptors following Theo treatment might act as an adaptive, compensatory process to return the heart to normal contractile activity and induce a homeostatic situation in the stimulated heart. As was mentioned earlier, up-regulation of A₁ receptors was also induced by thyroid hormones and by catecholamines /27,41/. These hormones also induce increases in heart rate. It seems that the effects and the mechanisms that cause Theo to sensitize the receptors are similar to those in hyperthyroidism or in stress conditions such as ischemia or hypoxia, in which the levels of catecholamines increase. Recent studies have indicated that Theo has potential as an anti-ischemic medication. Barbour *et al* /42/ and Heller *et al*. /19/ showed that administration of varying doses of Theo prior to exercise produces a clinically significant and concentration-dependent improvement in patients with chronic stable coronary artery disease. Similar results were obtained with bamiphylline /20/. Moreover, Alboni *et al*. /43/ showed that Theo is an effective therapy in patients with symptomatic atrial fibrillation and slow ventricular response. The therapeutic effect of Theo in myocardial ischemia and coronary artery diseases might also occur through up-regulation of A₁-adenosine receptors. This hypothesis deserves further analysis.

Acknowledgments

We are indebted to Mrs. T. Zinman and A. Isaac for their valuable technical assistance. This research is supported by Grant no. 93-222 from the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel and from the Otto Meyerhoff Drug Receptor Center at Bar-Ilan University.

References

1. Belardinelli L, Wu SN, Visentin S. Adenosine regulation of cardiac electrical activity. In: Zipes DP, Jalife J, editors. *Cardiac Electrophysiology: From Cell to Bedside*. WB Saunders; 1990. p. 284-290.
2. Berne RM. The role of adenosine in the regulation of coronary blood flow. *Circ Res*. 1980; 47:807-813. [PubMed: 6254686]
3. Ely SW, Berne RM. Protective effects of adenosine in myocardial ischemia. *Circulation*. 1992; 85:893-904. [PubMed: 1537125]
4. Ogilby JD, Jaekyeong H, Iskandrian AS. Effect of adenosine on coronary blood flow and its use as diagnostic test for coronary artery disease. *Cardiol Res*. 1993; 27:48-54.
5. Olsson RA, Pearson JD. Cardiovascular purinoceptors. *Physiol Rev*. 1990; 70:761-845. [PubMed: 2194223]
6. Belardinelli L, Linden J, Berne RM. The cardiac effects of adenosine. *Prog Cardiovasc Dis*. 1989; 32:73-97. [PubMed: 2664879]
7. Endo M, Maruyama M, Taira N. Modification by islet-activating protein of direct and indirect inhibitory actions of adenosine on rat atrial contraction in relation to cyclic nucleotide metabolism. *J Cardiovasc Pharmacol*. 1983; 5:131-142. [PubMed: 6186849]
8. Fu LX, Liang QM, Waagstein F, Hoebeke, Sylven C, Jansson E, Sotonyi P, Hjalmarson A. Increase in activity rather than in amount of Gi- α in failing human heart with dilated cardiomyopathy. *Cardiovasc Res*. 1992; 26:950-955. [PubMed: 1336712]
9. Leung EC, Johnston CI, Woodcock EA. Demonstration of adenylate cyclase coupled adenosine receptors in guinea pig ventricular membranes. *Biochem Biophys Res Comm*. 1983; 110:209-215.
10. Liang BT, Donovan LA. Differential sensitization of adenosine receptor mediated inhibition of cardiac myocyte contractility and adenylate cyclase activity. *Circ Res*. 1990; 67:406-414. [PubMed: 2376079]
11. Linden J, Hollen CE, Patel A. The mechanism by which adenosine and cholinergic agents reduce contractility in rat myocardium: Correlation with cyclic adenosine monophosphates and receptor densities. *Circ Res*. 1985; 56:728-735. [PubMed: 2581720]
12. Londos C, Wolff J. Two distinct adenosine-sensitive sites on adenylate cyclase. *Proc Natl Acad Sci USA*. 1977; 74:5482-5486. [PubMed: 271970]
13. Xu J, Tong H, Hurt CM, Pelleg A. Endogenous adenosine, A₁ adenosine receptor and pertussis toxin sensitive guanine nucleotide binding protein mediate hypoxia induced AV nodal conduction block in guinea pig heart in vivo. *Cardiovasc Res*. 1993; 27:134-140. [PubMed: 8458024]
14. Belardinelli L, Isenberg G. Isolated atrial myocytes: adenosine and acetylcholine increase potassium conductance. *Am J Physiol*. 1983; 244:13, H734-H737.
15. Belardinelli L, Giles WR, West A. Ionic mechanism of adenosine actions in pacemaker cells from rabbit heart. *J Physiol Lond*. 1988; 405:615-633. [PubMed: 2855644]
16. Kurachi Y, Nakajima T, Sugimoto T. On the mechanism of activation of muscarinic K⁺ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins. *Pflugers Arch*. 1986; 407:264-274. [PubMed: 2429251]
17. Lee HT, Thompson CI, Linden J, Belloni FL. Differential sensitization of cardiac actions of adenosine in rats after chronic theophylline treatment. *Am J Physiol*. 1993; 264:H1634-H1643.
18. Jacobson KA, Van Galen PJM, Williams M. Adenosine receptors: Pharmacology, structure-activity relationships and therapeutic potential. *Experientia*. 1983; 39:1347-1349. [PubMed: 6653721]
19. Heller GV, Barbour MM, Dweik RB, Coming JJ, McClellan JR, Garber CE. Effects of intravenous theophylline on exercise-induced myocardial ischemia. *J Am Coll Cardiol*. 1993; 21:1075-1097. [PubMed: 8459060]
20. Gaspardone A, Crea F, Iamele M, Tomai F, Versaci F, Pellergrino A, Chiariello L, Gioffre PA. Bamiphylline improves exercise-induced myocardial ischemia through a novel mechanism of action. *Circulation*. 1993; 88:502-508. [PubMed: 8339412]
21. Zhang Y, Weils JN. The effects of chronic caffeine administration on peripheral adenosine receptors. *J Pharmacol Exp Ther*. 1990; 254:757-763. [PubMed: 2395110]

22. Fastbom J, Fredholm BB. Effect of long-term theophylline treatment on adenosine A₁ receptors in rat brain: autoradiographic evidence for increased receptor number and altered coupling to G-proteins. *Brain Res.* 1990; 507:195–199. [PubMed: 2110844]
23. Murray TF. Up-regulation of rat cortical adenosine receptors following chronic administration of theophylline. *Eur J Pharmacol.* 1982; 82:113–114. [PubMed: 6290230]
24. Ramkumar V, Bumgarner JR, Jacobson KA, Stiles L. Multiple components of the A₁ adenosine receptor-adenylate cyclase system are regulated in rat cerebral cortex by chronic caffeine ingestion. *J Clin Invest.* 1988; 82:242–247. [PubMed: 3392208]
25. Wu SN, Linden J, Visentin S, Boykin M, Belardinelli L. Enhanced sensitivity of heart cells to adenosine and up-regulation of receptor number after treatment of guinea pigs with theophylline. *Circ Res.* 1989; 65:1066–1077. [PubMed: 2551526]
26. Liang BT, Donovan LA. Sensitization of cardiac A₁ adenosine receptor-linked physiologic responses. *Circulation.* 1989; 80:II-444. [Abstract].
27. El-Ani D, Jacobson KA, Shainberg A. Characterization of adenosine receptors in intact cultured heart cells. *Biochem Pharmacol.* 1994; 48:727–735. [PubMed: 8080445]
28. Brik H, Shainberg A. Thyroxine induces transition of red towards white muscle in cultured heart cells. *Basic Res Cardiol.* 1990; 85:237–246. [PubMed: 2166497]
29. Disatnik MH, Shainberg A. Regulation of β -adrenoceptors by thyroid hormone and Amiodarone in rat myocardial cells in culture. *Biochem Pharmacol.* 1991; 41:1039–1044. [PubMed: 1672592]
30. Waisberg M, Shainberg A. Characterization of muscarinic cholinergic receptors in intact myocardial cells in vitro. *Biochem Pharmacol.* 1992; 43:2327–2334. [PubMed: 1610397]
31. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72:248–254. [PubMed: 942051]
32. Green A, Johnson JL, Milligan G. Down regulation of G_i sub-types by-prolonged incubation of adipocytes with an A₁ adenosine receptor agonist. *J Biol Chem.* 1990; 265:5206–5210. [PubMed: 2108170]
33. Hadcock JR, Port JD, Malbon CC. Cross-regulation between G-protein mediated pathways: activation of the inhibitory pathway of adenylyl cyclase increases the expression of β_2 -adrenergic receptors. *J Biol Chem.* 1991; 266:11915–11922. [PubMed: 1646818]
34. Hoffman BB, Chang H, Dall'Aglia E, Reaven GM. Desensitization of adenosine receptor-mediated inhibition of lipolysis. *J Clin Invest.* 1986; 78:185–190. [PubMed: 3013937]
35. Longabaugh JP, Didsbury J, Spiegel A, Stiles GL. Modification of the rat adipocyte A₁ adenosine receptor adenylyl cyclase system during chronic exposure to an A₁ adenosine receptor agonist: alterations in the quantity of G_{sα} and G_{iα} are not associated with changes in their mRNAs. *Mol Pharmacol.* 1989; 36:681–688. [PubMed: 2511426]
36. Liang BT, Hirsch AJ. Homologous sensitization of embryonic chick atrial myocytes to adenosine: mediation by adenosine A₁ receptor and guanine nucleotide binding protein. *Cardiovasc Res.* 1993; 27:102–110. [PubMed: 8458019]
37. Shryock JA, Patel A, Belardinelli L, Linden J. Down regulation and desensitization of A₁-adenosine receptors in embryonic chicken heart. *Am J Physiol.* 1989; 256:H321–H327. [PubMed: 2916668]
38. McKenzie JE, Steffen RP, Haddy FJ. Effect of theophylline on adenosine production in the canine myocardium. *Am J Physiol.* 1987; 252:H204–H210. [PubMed: 3101516]
39. Fredholm BB. Cardiovascular and renal actions of methylxanthines. In: Spiller, AG., editor. *The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects*. New York: Liss; 1984. p. 303-330.
40. Fredholm BB. On the mechanism of action of theophylline and caffeine. *Acta Med Scand.* 1985; 217:149–153. [PubMed: 2986418]
41. El-Ani, D., Jacobson, KA., Shainberg, A. Regulation of adenosine receptors in cultured heart cells. In: Sideman, S., Beyar, R., editors. *Molecular and Subcellular Cardiology: Effects on Structure and Function*. Plenum Publishing Corp; 1995. p. 205-215.

42. Barbour MM, Garber CE, Ahlberg AW, Clouteir DJ, McClellan JR, Heller GV. Effects of intravenous theophylline on exercise-induced myocardial ischemia: II. A concentration-dependent phenomenon. *J Am Coll Cardiol.* 1993; 22:1155–1158. [PubMed: 7691911]
43. Alboni P, Paparella N, Cappatto R, Pirani R, Yiannacoupulu P, Antonioli GE. Long-term effects of theophylline in atrial fibrillation with a slow ventricular response. *Am J Cardiol.* 1993; 72:1142–1145. [PubMed: 8237803]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

Effect of theophylline on $[^3\text{H}]\text{CPX}$ binding. **A.** Five day-old cardiocytes were treated with 50 μM Theo. Specific binding of $[^3\text{H}]\text{CPX}$ was determined following 84 hours. The cardiocytes were exposed to the indicated concentrations of the radioligand as described in Materials and Methods. Each value is the mean $\pm$ S.D. of triplicate determinations from a representative experiment of sister cultures. **B.** Scatchard plot of specific $[^3\text{H}]\text{CPX}$ binding of Fig. 1. $K_d = 0.19$ and 0.18 nM, and $B_{\text{max}} = 20.5$ and 28 fmol/dish in control and Theo treated cells, respectively.

Fig. 2.

Dose response of theophylline on $[^3\text{H}]$ CPX binding and heart rate. **A.** Four day-old cardiocytes were treated with the indicated concentrations of Theo for 5 days. Specific binding of $[^3\text{H}]$ CPX (●) and heart rate (□) were then determined. The results are expressed as means $\pm$ S.D. of at least triplicate ($[^3\text{H}]$ CPX binding) or 5 determinations (heart rate) from a representative experiment. **B.** The relationship between $[^3\text{H}]$ CPX binding and heart rate of Fig. 2A. The correlation coefficient, $r = 0.941$, was obtained using Spearman's test.

Fig. 3.

Contractile responses to theophylline. Four day-old cardiocytes were treated with the following concentrations of Theo for 5 days: **a.** control, **b.** 0.1 μM , **c.** 1.0 μM , **d.** 100 μM . Contractility (beats/min) measured under a phase contrast microscope was 42 ± 5 , 67 ± 7 , 71 ± 7 and 72 ± 6 , respectively. The results are from a representative experiment that was measured during 10 sec from one cardiocyte per dish. For every treatment at least 5 cells from different culture-dishes were monitored.

Fig. 4.

Effect of cAMP on [^3H]CPX binding. **A.** Four day-old cardiocytes were treated with 100 μM dibutyryl-cAMP. Specific binding of [^3H]CPX was determined following 96 hours. The cardiocytes were exposed to the indicated concentrations of the radioligand as described in Materials and Methods. Each value is the mean $\pm$ S.D. of triplicate determinations from a representative experiment of sister cultures. **B.** Scatchard plot of specific [^3H]CPX binding of Fig. 4A. $K_d = 0.12$ and 0.13 nM, and $B_{\text{max}} = 24.4$ and 36.0 fmol/dish in control and cAMP treated cells, respectively.

Fig. 5.

Dose response of cAMP on [3 H]CPX binding and heart rate. Five day-old cardiocytes were treated with the indicated concentrations of dibutyl-cAMP for 3 days. Specific binding of [3 H]CPX (A) and myocyte contractility following 100 μ M dibutyl-cAMP (B) were determined. The results are expressed as means $\pm$ S.D. of at least triplicate ([3 H]CPX binding) or 5 determinations (heart rate) from a representative experiment.

TABLE 1

The effects of Theo on cardiac cells

	Control	Theo	P
CK (U/dish)	0.70 ± 0.08	0.64 ± 0.02	0.28
Protein (mg/dish)	1.10 ± 0.10	1.02 ± 0.13	0.45
Rate of contraction (beats/min)	42 ± 3	66 ± 2.4	0.004
Cell number (×10 ⁴)	90 ± 6	78 ± 6	0.07
Adenosine receptors (fmol/dish)	21.9 ± 2.6	52.0 ± 1.49	0.001

Four day-old cardiocytes were treated with 100 µM Theo for 5 days. The levels of specific ³[H]CPX binding were then determined. Sister cultures were homogenized for measurement of proteins and enzyme activities. Rate of contractions (beats/min) and cell number were measured on separate groups of cells. The results are expressed as means ± SD of at least triplicate determinations from a representative experiment. P - values of paired Student's t-test.

TABLE 2

The effects of cAMP on cardiac cells

	Control	cAMPdB	P
CK (U/dish)	0.70 ± 0.08	0.80 ± 0.10	0.25
Protein (mg/dish)	1.33 ± 0.14	1.13 ± 0.14	0.16
Rate of contraction (beats/min)	80 ± 20	180 ± 31	0.009
Cell number (×10 ⁴)	99 ± 7	110 ± 9	0.17
Adenosine receptors (fmol/dish)	24.4 ± 2.1	36.0 ± 2.7	0.05

Four day-old cardiocytes were treated with 100 µM cAMPdB for 5 days. The levels of specific ³[H]CPX binding were then determined. Sister cultures were homogenized for measurement of proteins and enzyme activities. Rate of contractions (beats/min) and cell number were measured on separate groups of cells. The results are expressed as means ± SD of at least triplicate determinations from a representative experiment. P - values of paired Student's t-test.