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EFFECTS OF *d*- AND *l*-PROPRANOLOL ON THE RESPONSIVENESS OF HUMAN FIBROBLASTS TO CHOLERAGEN AND PROSTAGLANDIN E_1

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Abstract—The cAMP content of cultured human fibroblasts was increased by incubation of the cells with choleragen, prostaglandin E_1 (PGE_1), or isoproterenol. Propranolol (80 μM) markedly reduced the elevation in intracellular cAMP induced by choleragen or isoproterenol but increased the accumulation of cAMP secondary to PGE_1. ("Propranolol" refers to the racemic mixture; when either isomer alone was used, the appropriate designation is given.) Propranolol prolonged the delay in choleragen responsiveness, normally apparent in human fibroblasts; the effects of propranolol on choleragen and PGE_1 occurred in the presence or absence of 3-isobutyl-l-methylxanthine and were observed on both intracellular and extracellular cAMP. *d*- And *l*-propranolol were equally effective with choleragen and PGE_1, whereas *l*-propranolol was much more effective than *d*-propranolol in blocking the action of isoproterenol. Propranolol had no significant effect on the activation of adenylate cyclase by choleragen or PGE_1. Butoxamine and dichloroisoproterenol did not decrease cAMP accumulation induced by choleragen but enhanced the response to PGE_1. A lipid-soluble anesthetic, tetracaine, also increased the accumulation of intracellular cAMP due to PGE_1 but inhibited the response to choleragen. These data indicate that propranolol exerts effects independent of its β-adrenergic blocking activity on agonist-induced changes in cAMP metabolism of human fibroblasts. In view of the concentrations of propranolol at which these "nonspecific" effects were observed, and based on similarities to actions of other drugs, it appears that some of the toxic and/or therapeutic effects of the drug may result from "membrane-stabilizing" properties and/or effects on microtubule assembly and may be unrelated to β-adrenergic blockade.

Choleragen, isoproterenol, and prostaglandin E_1 (PGE_1) activate adenylate cyclase in cultured human fibroblasts [1, 2]. Isoproterenol is believed to exert its effects through interaction with a β-adrenergic receptor, which is distinct from adenylate cyclase [3]. PGE_1 effects also appear to be mediated through a surface receptor that apparently is unrelated to that for isoproterenol [4]. The events following binding of these agents that lead to activation of adenylate cyclase are still unknown. In the case of choleragen, the initial step appears to be the binding of the B protomer of the toxin to the cell surface receptor, the ganglioside G_{M1} [5, 6]. Several investigators have suggested that mobility of the toxin within the membrane may be critical for the subsequent release of the active A_1 subunit from the toxin and its activation of adenylate cyclase through an NAD-dependent ADP-ribosylation of the cyclase or of a regulatory protein [7–11].

The importance of membrane function and integrity for choleragen activation of adenylate cyclase has been investigated using agents that alter the behavior of microtubules and microfilaments and inhibit "capping" and "patching" on the cell surface [12]. Propranolol,† in addition to its stereospecific β-adrenergic blocking activity, has nonstereospecific effects on cells believed to result from an "anesthetic" action on membranes [13–15]. The effects of propranolol on the actions of choleragen and hormones were investigated further in cultured human fibroblasts. These cells, which have a β-adrenergic receptor, respond to isoproterenol, PGE_1, and choleragen with increases in intracellular cAMP [1, 2]. The capacities of these cells to respond to isoproterenol and PGE_1 are independently regulated during growth in subculture and during incubation with choleragen [1]. In contrast to the stereospecificity of its β-blocking action in which *l*-propranolol is much more effective than *d*-propranolol in inhibiting effects of isoproterenol, the present study demonstrates that *d*- and *l*-propranolol are equipotent in inhibiting choleragen action. Furthermore, *d*- and *l*-propranolol are equally effective in increasing intracellular and extracellular accumulation of cAMP produced by PGE_1. Other β-blockers were effective only on PGE_1 action. Based on the differential effects of the β-blockers and their similarities to drugs possessing membrane-stabilizing action

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† "Propranolol" refers to the racemic mixture; when either isomer alone was used, the appropriate designation is given.

and/or effects on microtubule assembly, it would appear that propranolol and other β-blockers produce both therapeutic and toxic effects by any of several mechanisms.

EXPERIMENTAL PROCEDURE

Human foreskin fibroblasts were maintained in monolayer cultures in 250-ml flasks (Falcon Plastics) in Eagle's basal medium supplemented with Earle's salts, 10% fetal calf serum, and 2 mM glutamine. Cells were removed from flasks by brief (<5 min) treatment with 0.125% trypsin in Dulbecco's phosphate-buffered saline without Ca^{2+} and Mg^{2+} (Grand Island Biological Co., Grand Island, NY). Subcultures were initiated in Optilux tissue culture dishes (100 × 20 mm) that contained 10 ml growth medium and were incubated at 37° in a Forma CO_2 incubator as described previously [1]. Medium was changed on days 3 and 6. On day 7, when the cultures were essentially confluent, the growth medium was removed; the cultures were washed twice with 2 ml of Hanks' medium, and 10 ml of Hanks' medium was added to each dish. Choleragen, isoproterenol, or PGE_1 was then added as indicated, and the cells were incubated at 37° for 60 min. Propranolol was added at the same time as the agonist, i.e. at zero time, unless otherwise stated. To terminate incubations, the medium was rapidly aspirated and 2 ml of 5% trichloroacetic acid were added to each dish. cAMP content of the fibroblasts was measured by the protein binding assay of Gilman [16]. In some experiments, in which cAMP content of the medium was determined, the medium was rapidly aspirated and brought to 5% trichloroacetic acid. cAMP was then isolated and assayed as described previously [17]. cAMP is expressed as total cAMP in the medium per mg cell protein. Data for cAMP content are reported in tables and figures as mean ± S.D. of values from triplicate incubations.

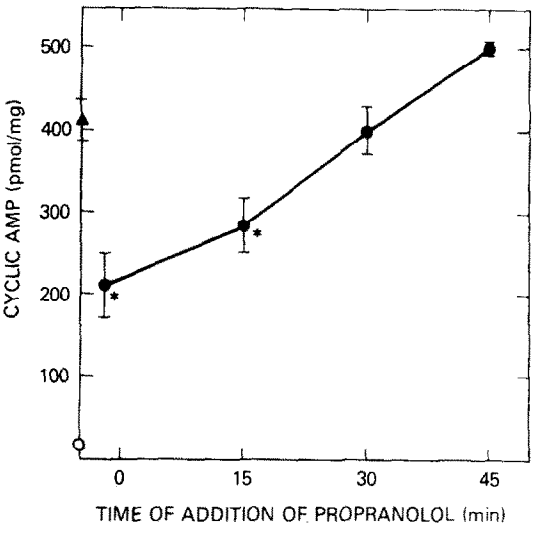

Fig. 2. Effect of time of addition of propranolol on the accumulation of cAMP secondary to choleragen. Cells were incubated for 60 min with choleragen (0.2 μg/ml) as described in Experimental Procedure. *d,l*-Propranolol (final concentration, 80 μM) was added 2 min before or 15, 30 or 45 min after choleragen (●). The cAMP content of cells incubated in medium alone and in medium with 80 μM propranolol was 16 (○) and 19 pmoles/mg protein respectively. The cAMP content of cells incubated with choleragen but without propranolol (▲) was 411 ± 27. Data are means ± S.D. of values from triplicate incubations. An asterisk indicates $P < 0.05$, compared to cells incubated with choleragen, without propranolol.

The NAD glycohydrolase and ADP-ribosyltransferase activities of choleragen were determined as described previously [8, 9]. Adenylate cyclase activity was measured by the method of Krishna *et al.* [18] as modified by Manganiello and Vaughan [19]. Protein was determined by the method of Lowry

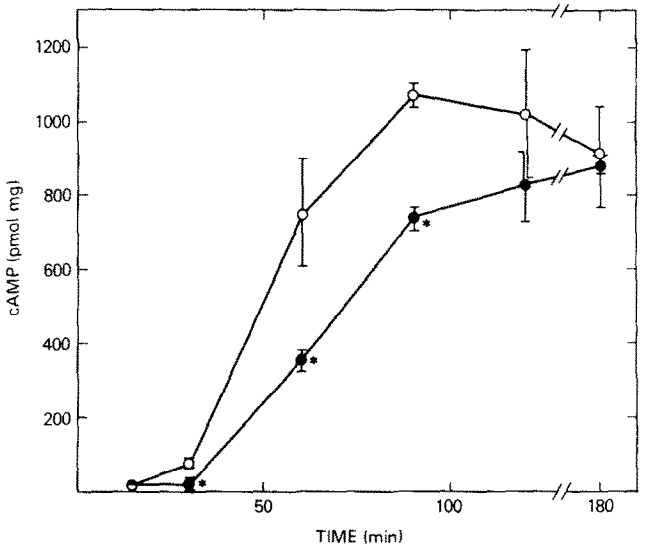

Fig. 1. Effect of propranolol on the time course of cAMP accumulation due to choleragen. Cells were incubated with (●) or without (○) 80 μM propranolol, which was added 5 min before choleragen, 0.04 μg/ml. After incubation with toxin for the times indicated, cell cAMP content was determined. Data are means ± S.D. of values from triplicate incubations. An asterisk indicates $P < 0.05$, compared to cells incubated with choleragen, without propranolol.

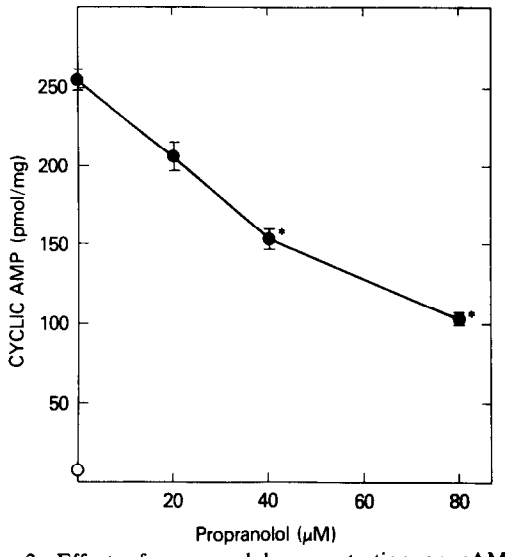

Fig. 3. Effect of propranolol concentration on cAMP accumulation due to choleragen. Cells were incubated with choleragen (0.04 μg/ml) for 60 min. Propranolol, at the indicated concentrations, was added 5 min prior to choleragen. The cAMP content of cells incubated in medium alone was 8 pmoles/mg protein (○). The cAMP content of cells incubated with choleragen but without propranolol was 255 ± 15 (●). Data are means ± S.D. of values from triplicate incubations. An asterisk indicates $P < 0.05$, compared to cells incubated with choleragen, without propranolol.

et al. [20]. Binding of [^{125}I]choleragen, prepared by the method of MacFarlane [21], was measured as described previously [22].

Materials. The sources of the materials used in the adenylate cyclase, ADP-ribosyltransferase, and NAD glycohydrolase assays have been noted [8, 9, 23]. Choleragen was purchased from Schwarz/Mann (Orangeburg, NY), tetracaine and isoproterenol from Sigma Chemical Co. (St. Louis, MO), butoxamine from Burroughs Wellcome, (Research Triangle Park, NC), propranolol from Ayerst (New York, NY), alprenolol from Hässle (Goteborg, Sweden) and dichloroisopropylnoradrenaline from ICN (Plainview, NY). PGE_1 was a gift of Dr. J. E. Pike (The Upjohn Co. Kalamazoo, MI).

RESULTS

Incubation of fibroblasts with choleragen resulted in an increase in intracellular cAMP (Fig. 1). Addition of 80 μM propranolol 5 min prior to choleragen markedly increased the delay in accumulation of cAMP (Fig. 1). In cells incubated with choleragen for 60 min, the addition of propranolol 15 min after the toxin decreased cAMP accumulation but to a lesser extent than it did when added 2 min before the toxin (Fig. 2). Propranolol added 30 or 45 min after toxin did not diminish the cAMP content of cells incubated for 60 min with choleragen (Fig. 2). Inhibition was demonstrable with 20 μM propranolol, and 80 μM decreased cAMP accumulation by about 50 per cent (Fig. 3). The effect of propranolol was observed on both intracellular and extracellular cAMP, in the presence and absence of 3-isobutyl-l-methylxanthine (IBMX) (Table 1). In the presence of higher concentrations of propranolol, the fibroblasts became rounded and no longer excluded trypan blue. Incubation for up to 90 min with 80 μM propranolol did not affect either cell morphology or dye exclusion.

In cells incubated with PGE_1, the cAMP content was markedly elevated at 15 min and declined thereafter but was still ~ten times basal after 60 min (Fig. 4). In the presence of 80 μM propranolol, the effects of PGE_1 on cAMP more than doubled at 15, 30 or

Table 1. Effect of propranolol (Pro) in the presence of choleragen (toxin) and/or IBMX on cAMP in cells and medium*

Additions			cAMP content (pmoles/mg protein)	
Toxin	IBMX	Pro	Cells	Medium
0	0	0	13.4 ± 1.4	19.7 ± 2.6
0	0	+	12.2 ± 1.6	24.3 ± 5.3
0	+	0	20.0 ± 2.7	47.5 ± 21.2
0	+	+	20.8 ± 2.4	34.6†
+	0	0	830 ± 111	985 ± 221
+	0	+	530 ± 51.1‡	351 ± 95.3‡
+	+	0	1240 ± 62.9	919 ± 113
+	+	+	965 ± 51.5‡	454 ± 11.2‡

* Cells were incubated with or without 80 μM propranolol for 5 min. Choleragen, (0.01 μg/ml) and/or 100 μM IBMX were added, as indicated, and incubations were terminated 60 min later. Data are means ± S.D. of values from triplicate incubations.

† The medium from only one dish was analyzed in the experiment.

‡ $P < 0.05$, compared to incubations in the absence of propranolol.

Table 2. Effects of *d*- and *l*-propranolol in the presence of PGE_1 on cAMP in cells and medium*

Propranolol (80 μM)	cAMP content (pmoles/mg protein) Cells	Medium
None	330 ± 43	1.3 ± 13.3
d-Propranolol	905 ± 103†	109 ± 12.0†
l-Propranolol	875 ± 112†	121 ± 27.1†

* Cells were incubated in triplicate with or without propranolol for 5 min. PGE_1 (0.2 μg/ml) was added to all cells, and incubations were terminated 15 min later. The cAMP content of cells incubated without PGE_1 and antagonists was 3.6 ± 2.2. Data are means ± S.D. of values from triplicate incubations.

† $P < 0.05$, compared to incubations with PGE_1 alone.

Table 3. Effect of propranolol (Pro) in the presence of PGE_1 on cAMP in cells and medium*

Additions PGE	Pro	cAMP content (pmoles/mg protein) Cells	Medium
0	0	19.9 ± 1.1	36.7 ± 8.6
0	+	22.1 ± 4.5	38.2 ± 4.7
+	0	1430 ± 51.4	450 ± 24.4
+	+	2740 ± 515†	728 ± 219†

* Cells were incubated with 100 μM IBMX with or without 80 μM propranolol, as indicated, for 5 min. PGE_1 (0.3 μg/ml) or diluent was added and incubations were terminated 15 min later. Data are means ± S.D. of values from triplicate incubations.

† $P < 0.05$, compared to incubations with PGE_1 without propranolol.

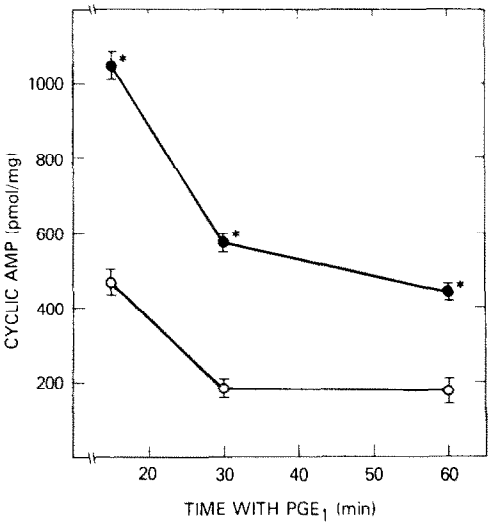

Fig. 4. Effect of propranolol on cAMP accumulation due to PGE_1. Cells were incubated with (●) or without (○) 80 μM propranolol for 5 min before addition of PGE_1, (0.2 μg/ml). At the indicated times thereafter cAMP content was determined. Data are means ± S.D. of values from triplicate incubations. An asterisk indicates *$P < 0.05$, compared to cells incubated with PGE_1 without propranolol. Basal cAMP content was determined at 60 min and was 13.0 ± 6.6 without propranolol and 5.9 ± 2.4 with propranolol.

Table 4. Effects of *d*- and *l*-propranolol on the cAMP content of fibroblasts incubated with isoproterenol*

Propranolol (concn)	cAMP content (pmoles/mg protein)
None	32.0 ± 3
d-Isomer (10 μM)	33.5 ± 6.4
d-Isomer (100 μM)	29.0 ± 1.4
l-Isomer (10 μM)	10.0 ± 4.2†
l-Isomer (100 μM)	8.5 ± 2.1†

* Cells were incubated for 60 min with 2 μM isoproterenol and propranolol as indicated. The cAMP content of cells incubated without isoproterenol or propranolol was 6 pmoles/mg protein. Data are means ± S.D. of values from triplicate incubations.

† $P < 0.05$, compared to incubation without propranolol.

Table 5. Effects of (+)- and (−)-alprenolol and dichloroisoproterenol on responsiveness of fibroblasts to PGE_1*

Additions (80 μM)	cAMP content (pmoles/mg protein)
None	413 ± 32.5
(+)-Alprenolol	1020 ± 212†
(−)-Alprenolol	1080 ± 91.2†
Dichloroisoproterenol	1010 ± 119†

* Fibroblasts were incubated for 5 min with additions as indicated. PGE_1 (0.2 μg/ml) was added, and the incubation was continued for 15 min. The cAMP content of cells incubated without PGE_1 or antagonists was 9 pmoles/mg protein. Data are means ± S.D. of values from triplicate incubations.

† $P < 0.05$, compared to cells incubated with PGE_1 without antagonist.

Table 6. Effects of β-adrenergic antagonists on the responsiveness of fibroblasts to PGE_1 or choleragen*

Additions (80 μM)	Cell cAMP content (pmoles/mg protein) Plus PGE_1	Plus choleragen
None	202 ± 14	450 ± 51
Dichloroisoproterenol	588 ± 120†	419 ± 32.5
Butoxamine	839 ± 32.5†	363 ± 7.1
d-, *l*-Propranolol	747 ± 33.2†	200 ± 15.6†

* Fibroblasts were incubated for 5 min with antagonist additions as indicated. PGE_1 (0.2 μg/ml) or choleragen (0.04 μg/ml) was then added, and the incubations were continued for 15 or 60 min respectively. The cAMP content of cells incubated without PGE_1 or choleragen was 29.2 ± 20.4. Data are means ± S.D. of values from triplicate incubations.

† $P < 0.05$, compared to cells incubated with PGE_1 or choleragen without antagonist.

Table 7. Reversibility of the effects of propranolol on responsiveness to PGE_1 and choleragen*

Propranolol (80 μM)	Other additions	Cell cAMP content (pmoles/mg protein) Unwashed	Washed
0	None	10 ± 1.0	13.3 ± 4.0
+	None	9 ± 0.0	12.7 ± 0.6
0	PGE_1	565 ± 70.6	449 ± 35.0
+	PGE_1	1040 ± 230†	674 ± 74.4†
0	Choleragen	407 ± 28.5	262 ± 1.5
+	Choleragen	164 ± 13.6‡	323 ± 86.4

* Fibroblasts were incubated for 5 min at 37° with or without 80 μM propranolol. To some (unwashed) PGE_1 (0.2 μg/ml) or choleragen (0.2 μg/ml) was then added. Others were washed three times with 2 ml of Hanks' medium before addition of 5 ml of medium containing, where indicated, PGE_1 (0.2 μg/ml) or choleragen (0.2 μg/ml). Incubations were terminated 15 min after the addition of PGE_1 and 60 min after the addition of choleragen. Data are means ± S.D. of values from triplicate incubations.

† $P < 0.05$ vs PGE_1 without propranolol.

‡ $P < 0.05$ vs choleragen, unwashed, without propranolol.

60 min (Fig. 4); 20 μM propranolol was about one-half as effective as 80 μM (data not shown). Both *d*- and *l*-propranolol increased the intracellular and extracellular cAMP content of fibroblasts incubated with PGE_1 (Table 2). Propranolol also increased the intracellular and extracellular cAMP content of cells incubated with PGE_1 and IBMX (Table 3).

The accumulation of cAMP in response to isoproterenol was inhibited by propranolol, and the *l*-isomer was much more potent than the *d*-isomer (Table 4). Inhibition by the *l*-isomer was observed at much lower concentrations than those required to alter responses to choleragen (Fig. 3) or PGE_1. The *d*- and *l*-isomers of propranolol were equipotent in inhibiting the effects of choleragen (Fig. 5). With either isomer, inhibition was observed only when it was added early in the incubation. At a concentration of 80 μM, (+)- and (−)-alprenolol likewise produced similar effects (Table 5). Butoxamine and dichloroisoproterenol caused little decrease in cAMP in the presence of choleragen, but both increased the response to PGE_1 (Table 6). Dichloroisoproterenol (80 μM) had no effect on the cAMP content of cells incubated without choleragen or PGE_1 (data not shown).

When cells that had been incubated with pro-

Table 8. Effect of propranolol on activation of adenylate cyclase by choleragen (toxin) or PGE_1*

Expt.	Additions (concn)	Adenylate cyclase activity (pmoles·min^{-1}·mg^{-1})
1	None	11.5 ± 0.5
	Propranolol (80 μM)	10.0 ± 3.0
	Toxin (50 μg/ml)	36.8 ± 7.2
	Propranolol + toxin	40.7 ± 5.2
2	None	51.0 ± 1.7
	Propranolol (80 μM)	61.1 ± 2.3
	PGE_1 (50 μg/ml)	99.4 ± 6.0
	Propranolol + PGE_1	108 ± 5.4

* For each experiment, fibroblasts from twelve dishes were washed twice with 2 ml of 0.25 M sucrose/20 mM Tris (Cl^-), pH 7.5, scraped, sedimented by centrifugation (1000 *g*, 10 min), and homogenized (100 strokes in a Dounce homogenizer) in the same medium. The homogenate was centrifuged (20,000 *g*, 20 min). The sedimented particulate fraction was washed and suspended in 0.6 ml of the same buffer. In experiment 1, samples of particulate fraction (240 μg protein) were incubated with activated choleragen [previously incubated with 20 mM dithiothreitol and ovalbumin (1 mg/ml) for 10 min at 30°] in a total volume of 80 μl containing 1 mM NAD, 100 μM GTP, 10 mM phospho(enol)pyruvate, pyruvate kinase (8 units), 10 mM $MgCl_2$, 200 mM NaCl, and 10 mM Tris (Cl^-), pH 8.0. Assay solution (20 μl) was then added so that final concentrations of reagents were 1 mM [α-^{32}P]ATP (~150,000 cpm), 5 mM $MgCl_2$, 10 μM GTP, 1 mM cAMP, 3 mM theophylline, 2 mM dithiothreitol, 2 mM phospho(enol)pyruvate, pyruvate kinase (12 units/ml), and 25 mM Tris(Cl^-), pH 8.0. After 10 min at 30°, assays were terminated, and cAMP was isolated for radioassay as described previously [23]. In experiment 2, samples of particulate were assayed as described above with additions as indicated. Data are means ± S.D. of values from triplicate assays.

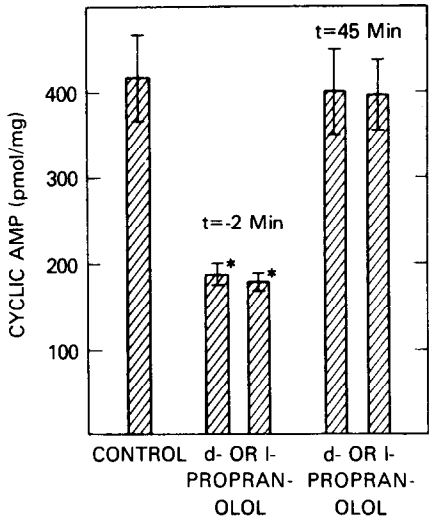

Fig. 5. Effects of *d*- and *l*-propranolol on cAMP accumulation due to choleragen. Cells were incubated with choleragen (0.2 μg/ml) for 60 min prior to determination of cAMP. *d*- or *l*-propranolol (80 μM) was added at the indicated times relative to the addition of choleragen. Data are means ± S.D. of values from triplicate incubations. An asterisk indicates $P < 0.05$, compared to cells incubated with choleragen, without propranolol.

pranolol for 5 min were washed before addition of PGE_1 or choleragen, only relatively small effects of the drug on responses to the two agonists were observed (Table 7). Propranolol had no effect on the activation of adenylate cyclase by PGE_1 or choleragen (Table 8), on the activity of cAMP phosphodiesterase in fibroblast homogenates, or on the ADP-ribosyltransferase activity of the toxin (data not shown). Tetracaine, a local anesthetic, also decreased the responsiveness of the fibroblasts to choleragen and enhanced the effects of PGE_1 on intracellular cAMP (Table 9).

DISCUSSION

In the studies reported here, propranolol modified the response of cultured human fibroblasts to hormones and choleragen. Stereospecific β-adrenergic blockade was produced with submicromolar concentrations of propranolol, whereas *d*- and *l*-propranolol in higher concentrations enhanced accumulation of cAMP in cells exposed to PGE_1 and inhibited cAMP accumulation in cells incubated with choleragen. A number of nonstereospecific effects of propranolol, which were produced with concentrations of drug higher than those necessary to achieve β-blockade, have been reported [13–15, 24–29]. These effects, which include inhibition of patching and capping on lymphocytes [15] and inhibition of rheumatoid factor secretion [29], have been attributed to the ability of propranolol to act as a membrane-stabilizing agent or to interact with membrane phospholipids. Butoxamine and dichloroisoproterenol, β-adrenergic antagonists that do not exhibit membrane-stabilizing effects, did not inhibit the response to choleragen but did enhance cAMP accumulation in the presence of PGE_1. In another study, butoxamine, unlike propranolol, did not interfere with the release of rheumatoid factor from lymphoid cells [29]. Tetracaine mimicked the effect of propranolol on both choleragen- and PGE_1-induced accumulation of cAMP in human fibroblasts, and it has been reported that tetracaine can enhance the effect of PGE_1 on cAMP content of some normal and transformed cells [30, 31].

Activation of adenylate cyclase by choleragen is believed to result from the NAD-dependent ADP-ribosylation of a protein component of the adenylate cyclase system catalyzed by the A_1 portion of the toxin [7–11]. Although the mechanism by which the A_1 peptide gains access to the adenylate cyclase is unclear, it has been reported that binding of toxin to lymphocytes is followed by patching and capping [11], and these processes may be involved in its action. Propranolol, which has been reported to inhibit patching and capping [15], could slow choleragen entry and reduce the rate or extent of adenylate cyclase activation.

The mechanisms whereby propranolol, butoxamine, dichloroisoproterenol, and tetracaine increased responsiveness to PGE_1 may not be related to membrane stabilization. Local anesthetics such as tetracaine have been reported not only to affect membrane stability and interact with membrane phospholipids but also to alter the organization of microtubules and microfilaments [31, 32]. Recently, Rudolph *et al.* [33] have demonstrated that in intact white cells colchicine and agents that affect microtubular assembly increase responsiveness to PGE_1. Based on differences in their nonstereospecific effects on responsiveness to choleragen and PGE_1, β-blocking agents may be divided into two categories. Those β-blocking agents that enhance responsiveness to PGE_1 affect microtubular organization; those that possess membrane-stabilizing action

Table 9. Effect of tetracaine on the responsiveness of human fibroblasts to PGE_1 and choleragen*

Additions (concn)	cAMP content (pmoles/mg protein) No tetracaine	Tetracaine (30 μM)
PGE_1 (0.2 μg/ml)	271 ± 73.5	662 ± 20.5†
Choleragen (0.04 μg/ml)	1270 ± 120	560 ± 149†

* Fibroblasts were incubated with PGE_1 for 15 min or with choleragen for 60 min with or without 30 μM tetracaine. cAMP content of cells incubated with no additions was 11 pmoles/mg protein. Data are means ± S.D. of values from triplicate incubations.

† $P < 0.05$, compared to incubations without tetracaine.

inhibit activation by choleragen. Propranolol, for example, might affect membrane stability and microtubular assembly; butoxamine, which does not affect membrane stability, might affect microtubule assembly.

The concentrations of propranolol necessary to produce nonstereospecific effects are much greater than the concentration of *l*-propranolol required for β-adrenergic blockade and are greater than those usually found in the serum of persons receiving propranolol orally in therapeutic dosage [34, 35]. Following intravenous administration, however, the propranolol concentration in plasma transiently reaches levels that are effective in membrane stabilization [36]. In addition, since propranolol can be concentrated in tissues [36, 37], and since individual differences in tissue uptake, metabolism and responsiveness do exist, cellular concentrations of the drug, particularly after chronic administration, may be within the range necessary for nonstereospecific effects. Alteration in oxyhemoglobin dissociation resulting from chronic administration and normalization of the P_{50} following withdrawal of the drug has been reported recently [28]. Psychotic episodes have occurred in some patients receiving propranolol in amounts often used for treatment of angina pectoris, i.e. ~200 mg/day [38–40]. Higher doses of propranolol have been used as a potential treatment for schizophrenia, with therapeutic effects reported at concentrations that are consistent with nonstereospecific effects [41]. In addition, some of the antiarrhythmic actions of propranolol and several propranolol derivatives are related to quinidine-like effects, which are independent of β-blockade [42, 43]. It seems very likely that some of the toxic and therapeutic effects of propranolol are unrelated to β-blockade and result from membrane stabilization or effects on microtubule assembly. Enhanced responsiveness to prostaglandins, as noted in the present study, may be one example of the latter. A better understanding of the multiple effects of this drug could result in more effective clinical usage if selective advantage were taken of the β-blocking or nonstereospecific effects of the drug.

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