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Stress Induced Desensitization of Lymphocyte β-Adrenoceptors in Young and Aged Rats

A. DE BLASI,[1] M. LIPARTITI, S. ALGERI, G. SACCHETTI, C. COSTANTINI, M. FRATELLI AND S. COTECCHIA

Istituto di Ricerche Farmacologiche, "Mario Negri," Via Eritrea, 62-20157 Milano, Italy

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DE BLASI, A , M LIPARTITI, S ALGERI, G SACCHETTI, C COSTANTINI, M FRATELLI AND S COTECCHIA. *Stress induced desensitization of lymphocyte β-adrenoceptors in young and aged rats.* PHARMACOL BIOCHEM BEHAV 24(4) 991–998, 1986 — The effects of different times of immobilization stress on intact lymphocyte β-adrenoceptors and plasma corticosterone were compared in 3-month and 24-month-old rats. In young animals after 30 min restraint ^{3}H-dihydroalprenolol specific binding was significantly reduced (61% of control value) and plasma corticosterone significantly raised (186% of control). The effect on β-adrenoceptors was due to changes in receptor number (Bmax) without any effect on affinity (K_D) In aged rats both effects were only seen after 180 min restraint and were less pronounced Isoproterenol treatment *in vitro* reduced β-adrenoceptors on lymphocytes This effect was less pronounced in lymphocytes from aged rats Corticosterone *in vitro* increased ^{3}H-dihydroalprenolol specific binding We therefore suggest that the decrease of β-adrenoceptors reflects an adaptive response to the stress-induced catecholamine release and that corticosterone could play a role in reversing this effect This adaptive response to stress seems to be impaired in aged animals

Stress β-Adrenoceptor desensitization Corticosterone Aging

STRESS is a common situation which induces several physiological responses such as activation of the pituitary-adrenocortical system. Some stress-induced changes can be interpreted as adaptive responses to this powerful stimulus For example a decrease in the number of β-adrenoceptors in rat brain after repeated stress has been considered a form of receptor adaptation to prevent some dangerous effects of persistent high levels of catecholamines [37].

Aging has an important influence on the physiological response to stress For example, the cardiovascular system's capacity to adapt to stress [27] and the rat's ability to regulate its body heat on exposure to cold stress [1] are impaired with aging Furthermore old rats lose their ability to cope with acute stress, because of a loss of adrenergic responsiveness and increased "shock" reaction [6]. The changes in receptor number [9,10] and the impaired receptor adaptability [6,17] observed with aging may be related to age-dependent alterations of stress-induced responses.

In young and aged rats we investigated the possibility that acute adaptation to stress [6,12] might involve receptor changes. The experimental model of the β-adrenergic receptor on intact lymphocytes was chosen for this purpose. In contrast with the scant information available about short-term receptor regulation in internal organs (heart, lung and brain) the acute regulation of β-adrenoceptors by homologous and heterologous agents *in vitro* has been extensively documented on different intact cell systems [19] including intact human lymphocytes [11,24] The finding that catecholamines and corticosteroids, the hormones which are released during stress, both modulated β-adrenergic receptors in these experimental systems [7,8] further sustained the present investigation.

METHOD

Young (3 months) and aged (24 months) male Sprague-Dawley CD-COBS rats (Charles River, Italy) maintained in standard laboratory conditions were used. Restraint stress procedures was induced as follows: 3 or 24-month-old rats were immobilized by wrapping them in flexible wire mesh (3×3 mm). In study A young rats were restrained for 0, 15, 30, 60, 90 and 180 min (5–6 animals per group). In study B young and aged rats were restrained for 0, 30, 90 and 180 min (5–6 animals per group). In order to minimize the influence of circadian rhythm, control and stressed young and aged rats were killed at random from 10:30 to 12:30 a.m.

When necessary (i.e., for *in vitro* studies or for Scatchard analysis) the blood from 7–10 rats was pooled

Indwelling Carotid Catheter

Rats were anesthetized with chloral hydrate (350 mg/kg IP) and an 8 cm long cannula (PP50, Portex, England) was inserted 2 cm into the left carotid artery and sutured at the muscle. Then the cannula was passed through the sub-

[1]Requests for reprints should be addressed to Antonio De Blasi, M D

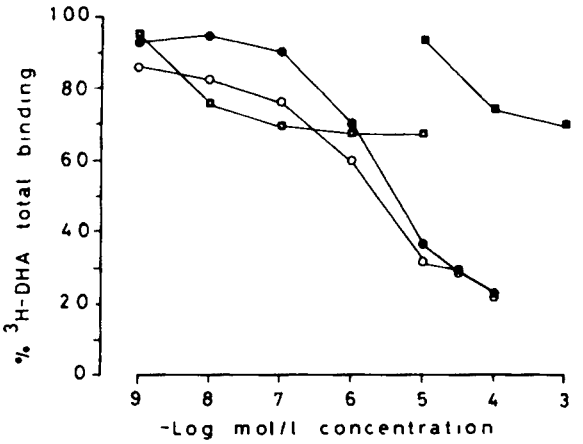

FIG 1 Inhibition of ^{3}H-dihydroalprenolol (^{3}H-DHA) binding by (−)-propranolol (○), (+)-propranolol (●), CGP 12177 (□), and isoproterenol (■) Each point is the mean of 3–5 separate experiments

TABLE 1

EFFECT OF RESTRAINT STRESS ON INTACT LYMPHOCYTE ^{3}H-DIHYDROALPRENOLOL SPECIFIC BINDING IN 3-MONTH-OLD RATS

Restraint time (minutes)	n	^{3}H-DHA specific binding (fmol/cell 10^6)	% of basal value
0	10	8 23 ± 1 00	100
15	3	6 63 ± 1 06	81
30	11	4 99 ± 0 55*	61
60	6	9 03 ± 1 02	110
90	9	8 76 ± 1 27	106
180	13	8 35 ± 0 56	101

Specific binding was measured as ^{3}H-dihydroalprenolol (^{3}H-DHA) binding inhibited by 30 μmol/l (±)-propranolol

Values are mean ± S E M of n rats

*Significantly different from basal value (time 0) at $p<0$ 05 (Dunnett's test)

cutaneous tissue in the cervical area and anchored with a stitch at the skin, 24–36 hours thereafter blood samples (about 1 ml) were collected into heparinized tubes (on ice) Samples from 7–8 rats were pooled and processed as described to isolate lymphocytes

Lymphocytes Isolation

Rats were killed by decapitation and trunk blood was collected into heparinized tubes taken on ice. Peripheral blood lymphocytes were prepared according to Oehler *et al* [28] with slight modification About 10 ml blood was diluted with 40 ml phosphate-buffered saline (PBS) (Eurobio), centrifuged at 200×g for 10 min and the supernatant discarded The cells, diluted to 40 ml with PBS, were carefully layered over 10 ml Ficoll-Hypaque (MSL 2000, Labtek) and centrifuged at 500×g for 45 min The cells layered at the interface of the Ficoll were carefully collected and washed with PBS To eliminate residual contaminating platelets the cells were centrifuged twice at 200×g for 10 min and the supernatant was discarded. Mononuclear cells were then incubated in RPMI-1640 medium supplemented with 20% fetal bovine serum (Gibco, Biocult, Glasgow, Scotland) for 45 min at 37°C in plastic Petri dishes (# 3003, Falcon Plastics, Oxnard, CA). Non-adherent cells were collected and washed with PBS The final cell preparation contained approximately 94% lymphocytes, 5% monocytes and <2% polymorphonuclear leucocytes.

^{3}H-Dihydroalprenolol Binding

Binding experiments on intact lymphocytes were performed according to Watanabe *et al* [41] with slight modifications The standard reaction mixture (1 ml) consisted of 50 mmol/l Tris-HCl buffer (pH 7 4 at 25°C), 85 mmol/l KCl, 10 mmol/l $MgCl_2$, 0 4 mmol/l ascorbic acid, 1 mmol/l EDTA, 10 μmol/l pargyline (incubation medium) and 2×10^6 intact lymphocytes The quite high concentration of KCl used to make the incubation medium isotonic did not affect the ^{3}H-DHA binding, as compared with ^{3}H-DHA binding in PBS or in Dulbecco's Modified Eagle Medium (data not presented) Incubation (15 min at 37°C) was stopped by the addition of 4 ml of ice-chilled Tris buffer followed by rapid filtration through Whatman GF/B glass fiber filters under vacuum and three additional 4 ml washes In saturation experiments 5–6 different concentrations of ^{3}H-dihydroalprenolol (S A 51 and 103 Ci/mmol, Amersham, England) ranging from 0 75 to 8 nmol/l were used In experiments where a single concentration of radioligand was used, 2 nmol/l was chosen and assayed at least in triplicate Non-specific binding, defined as ^{3}H-dihydroalprenolol binding in the presence of 30 μmol/l (±)-propranolol, was 30% of total binding at ^{3}H-dihydroalprenolol concentration of 2 nmol/l

^{3}H-CGP 12177 Binding

^{3}H-CGP 12177 [4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on-hydrochloride] binding to intact lymphocytes was determined as previously described [11] The standard reaction mixture (0 5 ml) consisted of Dulbecco's Modified Eagle Medium (pH 7 4 at 20°C) containing 20 mmol/l Hepes and 1 mg/ml bovine serum albumin (incubation medium) and $2\text{–}3\times10^6$ intact lymphocytes Incubation (18 hr at 4°C) was stopped by the addition of 10 ml cold PBS followed by rapid filtration through Whatman GF/C glass fiber filters under vacuum and one additional 10 ml wash ^{3}H-CGP 12177 (S A 43 Ci/mmol, Radiochemical Centre, Amersham, England) concentrations ranged from 0 30 to 9 nmol/l Non specific binding, defined as ^{3}H-CGP 12177 binding in the presence of 1 μmol/l (−)-propranolol, was 20% of total binding at $2\times K_D$

The lymphocytes used in this set of experiments were isolated from whole blood by performing all the steps at 4°C (adherence of monocytes to plastic was avoided) This modification (which did not change the yield of cells [11]) and the binding at low temperature was preferred because it gave more reliable results when measuring acute changes in ^{3}H-CGP 12177 binding to surface lymphocytes β-adrenoceptors [11] The characteristics of ^{3}H-CGP 12177 to intact lymphocytes are described in detail elsewhere [11]

Effect of Isoproterenol In Vitro

Lymphocytes resuspended at a density of $10\text{–}15\times10^6$

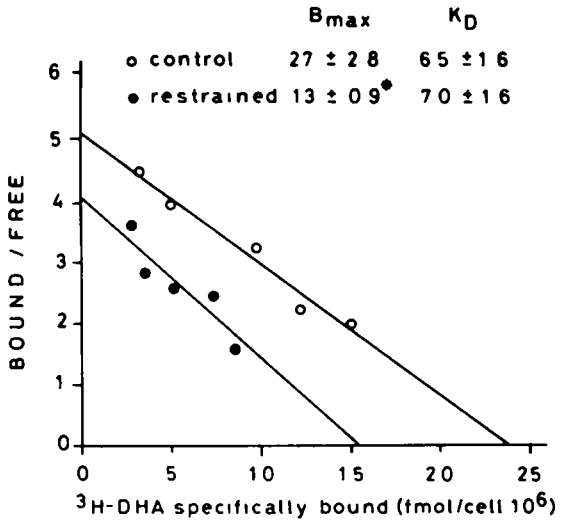

FIG 2 Effect of 30 min stress on lymphocyte total β-adrenoceptors Representative Scatchard plot of young control (○) and 30 min-restrained (●) rats Data are means±S E M of 3 separate experiments ^{3}H-dihydroalprenolol (^{3}H-DHA) specific binding was measured as described in Table 1 *$p<0$ 01, Student's t test

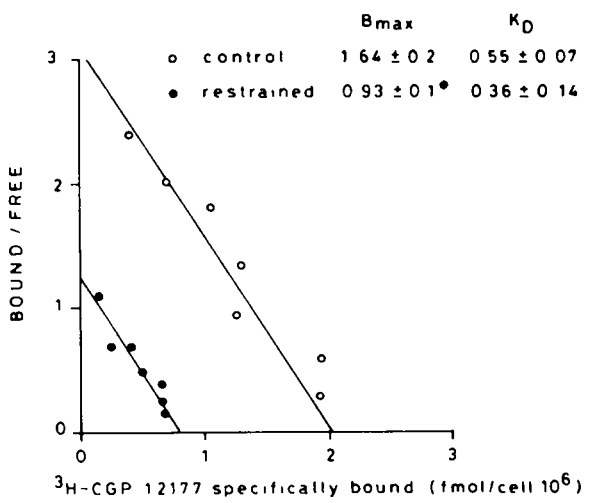

FIG 3 Effect of 30 min stress on lymphocyte surface β-adrenoceptors Representative Scatchard plot of young control (○) and 30 min (●) restraint rats Data are means±S E M of 3 separate experiments B_{max}=fmol/10^6 cell K_D=nM *$p<0$ 05, Student's t test

cells/ml were incubated without or with different concentrations of isoproterenol at 37°C. After 20 min cells were diluted with cold Tris buffer, rapidly chilled, washed twice with 50 ml cold Tris buffer and finally resuspended in incubation medium. Isoproterenol was protected from oxidation by using 0 01% (w/v) ascorbic acid.

Effect of Corticosterone In Vitro

The reaction was started by adding 0.5 ml of incubation medium containing 2×10^6 lymphocytes to 0.5 ml of incubation medium containing ^{3}H-dihydroalprenolol and different concentrations of corticosterone. After 15 min incubation at 37°C the reaction was stopped by rapid filtration. Corticosterone was dissolved in ethanol, which never exceeded 1% (vol/vol) in the binding sample. The same amount of solvent was added in the control sample. The effect of the hormone was observed on total binding without any change of non-specific binding.

Corticosterone Determination

In study B blood, collected in iced heparinized tubes, was centrifuged at 500×g for 10 min at 4°C, then stored at −70°C for corticosterone determination. Endogenous plasma corticosterone levels were determined spectrofluorometrically in 200 μl of plasma according to the method of Guillemin *et al* [18]

RESULTS

Figure 1 shows the characteristics of ^{3}H-dihydroalprenolol binding to rat intact lymphocytes. The binding of ^{3}H-dihydroalprenolol was inhibited stereospecifically by (−)- and (+)-propranolol, but the pattern of stereoselectivity was unusual (higher at 10^{-9}–10^{-7} mmol/l than at 10^{-6}–10^{-5} mmol/l [4]). Furthermore ^{3}H-dihydroalprenolol binding was displaced non-stereospecifically at higher propranolol concentrations [25]. Only a fraction (≈30%) of ^{3}H-dihydroalprenolol binding could be displaced by the antagonist CGP 12177 which, unlike ^{3}H-dihydroalprenolol and propranolol, is hydrophilic [32,36]. Taken together, these results indicate that ^{3}H-dihydroalprenolol binds to a heterogenous population of sites and that hydrophylic compounds (CGP 12177, isoprotenerol) selectively inhibited a single population of receptors. Since CGP 12177 probably binds only external sites [36] it is likely that the ^{3}H-dihydroalprenolol binding inhibited by CGP 12177 represents surface receptors. A fraction of ^{3}H-dihydroalprenolol binding not inhibited by CGP 12177 was stereospecific, although (−)-propranolol was only ≈3 times more potent than (+)-propranolol. Because of the major interactions of lipophilic drugs (such as ^{3}H-dihydroalprenolol and propranolol) with the membrane lipids, it was difficult to clarify the exact nature of these sites and assess whether they are non surface receptors or non-specific sites. However the existence in intact cells of a fraction of receptors sequestered in an environment not accessible to hydrophilic ligands has been previously reported [20, 36, 40].

Based on the above considerations in the present investigation β-adrenoceptors were measured as the ^{3}H-dihydroalprenolol binding inhibited by 30 μmol/l (±)-propranolol (total β-adrenoceptors) or by 1 μmol/l CGP 12177 (surface β-adrenoceptors).

As expected in intact cell systems [22,36] the agonist isoproterenol showed low affinity for β-adrenoceptors (Fig. 1).

Total β-adrenoceptors (see above) were measured on rat intact lymphocytes after immobilization stress The pooled findings of two experiments (studies A and B) on the effect of different times of immobilization stress on young rats are shown in Table 1. Because of the fast coagulation of the blood during collection in the 15-min restrained group, samples from only three animals in this group were available for lymphocyte preparation and binding determination. A signif-

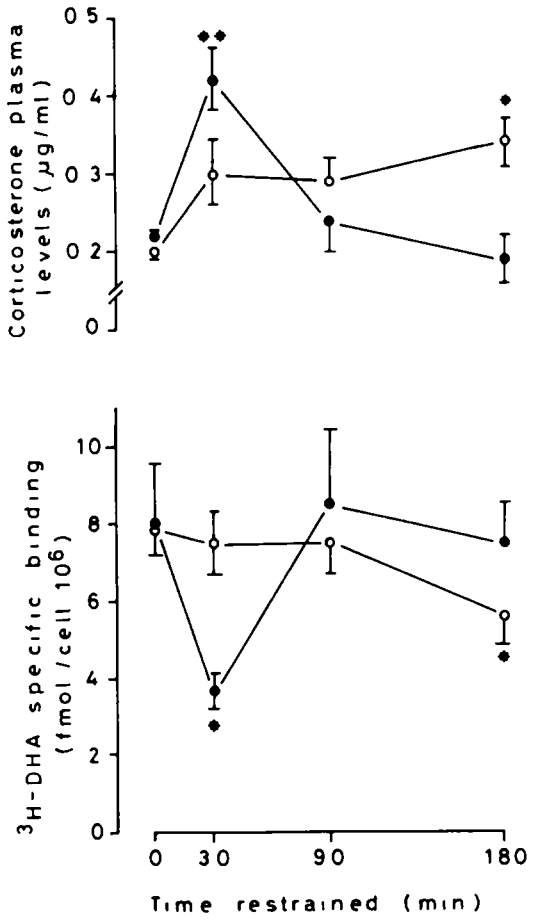

FIG 4 Effect of restraint stress on plasma corticosterone and ^{3}H-dihydroalprenolol (^{3}H-DHA) specific binding in young (●) and aged (○) rats Each point is the mean±S E M of 5–6 animals ^{3}H-dihydroalprenolol specific binding was measured as described in Table 1 Significant differences at $p<0$ 01 (**) and $p<0$ 05 (*) from basal value (time 0) (Dunnett's test)

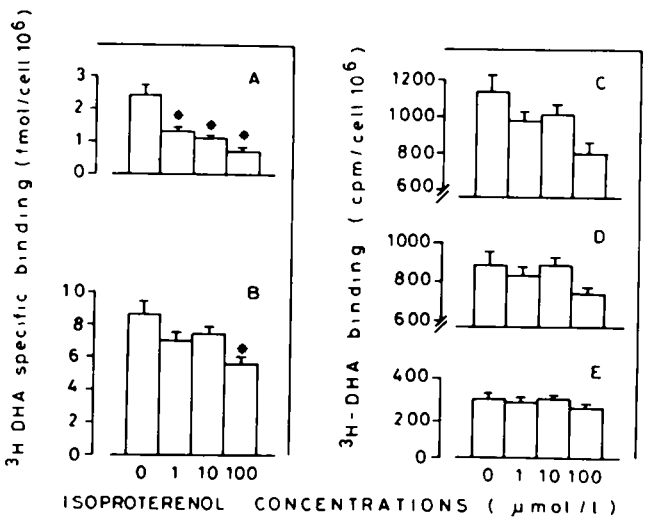

FIG 5 Effect of pretreatment with different concentrations of isoproterenol on lymphocytes from young control rats Shown are surface (A) and total (B) β-adrenoceptors and binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) alone (C) and in the presence of 1 μmol/l CGP 12177 (D) or 30 μmol/l (±)-propranolol (E) (n=4) Significant differences at $p<0$ 01 (*) from lymphocytes preincubated without isoproterenol (0) (Dunnett's test) For details see the Method section and Results section

icant decrease (39%) in ^{3}H-dihydroalprenolol specific binding was seen after 30 min stress, but after 60–120 min restraint receptor binding was the same as basal values The effect at 30 min remained statistically significant even when each experiment was analyzed separately

The decrease of ^{3}H-dihydroalprenolol binding observed reflected changes in receptor number. In saturation experiments the number of β-adrenoceptors was significantly reduced without any change in K_D after 30 min stress (Fig 2).

When the surface β-adrenoceptors were directly measured by ^{3}H-CGP 12177 specific binding, the number of surface β-adrenoceptors was markedly decreased in restrained rats (Fig 3). In this set of experiments we preferred to measure surface receptors by ^{3}H-CGP 12177 (instead of ^{3}H-dihydroalprenolol competed by unlabelled CGP 12177) because of the higher specific/total binding ratio which enabled us to perform reliable saturation experiments

Study B compared the effect of stress on lymphocyte β-adrenoceptors in young and old rats (Fig 4) Basal values of ^{3}H-dihydroalprenolol specific binding were similar in the two age groups However the decrease of β-adrenoceptors after 30 min restraint in young rats was not observed in aged animals The ^{3}H-dihydroalprenolol specific binding to lymphocytes of 24-month-old rats was unmodified after 30 and 90 min and a significant decrease was only seen after 180 min stress

Two major physiological events (i e , increased catecholamines and corticosterone secretion) occurring during stress may be related to the observed changes in β-adrenoceptors The effects of the β-adrenergic agonist isoproterenol and of corticosterone on both total and surface β-adrenoceptors were therefore investigated *in vitro*

Pre-exposure of rat living lymphocytes to isoproterenol caused a decrease in β-adrenoceptors, apparently due mainly to changes in surface receptors In fact ^{3}H-dihydroalprenolol binding inhibited by CGP 12177 (surface receptors) was markedly reduced (from 54 to 29% of control value) by all the isoproterenol concentrations tested (Fig 5A) A significant decrease of total β-adrenoceptors was only seen at the higher dose of isoproterenol (100 μmol/l) (Fig 5B) The effect of isoproterenol was due to a change in total binding with little effect on ^{3}H-dihydroalprenolol binding in the presence of either CGP 12177 (1 μmol/l) or propranolol (30 μmol/l) (Fig. 5, C, D, E) This was not because of competition from isoproterenol still present in the binding mixture, since very high concentration of isoproterenol is needed to inhibit ^{3}H-dihydroalprenolol binding (see Fig 1), and no persistent occupancy of the receptors by isoproterenol was found under similar experimental conditions [35]

The ability of isoproterenol to reduce surface β-adrenoceptors was impaired in lymphocytes from aged rats (Fig 6). *In vitro* preincubation with isoproterenol induced a slight (12–15%) decrease of surface β-adrenoceptors, and this effect reached statistical significance only at high drug concentrations

As shown in Fig 7, the presence of corticosterone con-

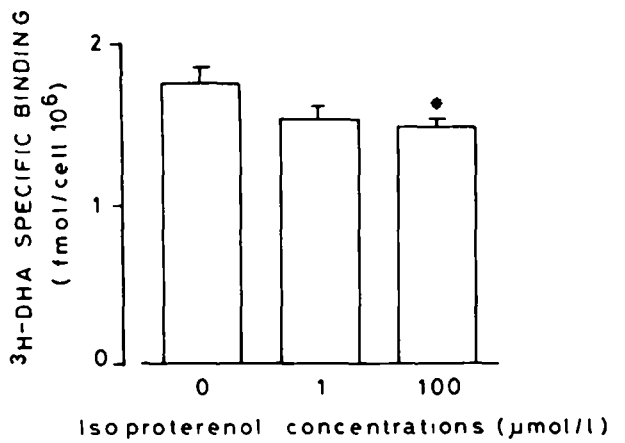

FIG 6 Effects of pretreatment with different concentrations of isoproterenol on lymphocytes from aged rats Shown are surface receptors, measured by ^{3}H-dihydroalprenolol binding competed by 1 μmol/l CGP 12177 (n=3) Significant difference at $p<0$ 05 (*) from lymphocytes preincubated without isoproterenol (0) (Dunnett's test)

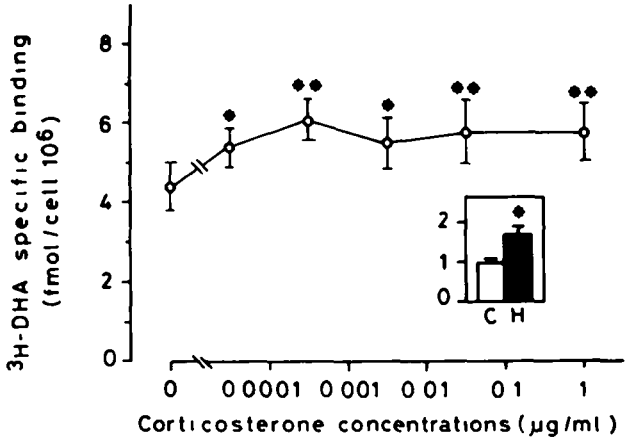

FIG 7 *In vitro* effect of corticosterone on lymphocyte total β-adrenoceptors (^{3}H-dihydroalprenolol (^{3}H-DHA) binding inhibited by 30 μmol/l (±)-propranolol) Each point is the mean±S E M of 5 experiments Significant differences at $p<0$ 01 (**) and $p<0$ 05 (*) from ^{3}H-dihydroalprenolol binding without corticosterone The inset shows surface β-adrenoceptors (^{3}H-dihydroalprenolol binding inhibited by 1 μmol/l CGP 12177) in the absence (C) or presence (H) of 0 5 μg/ml corticosterone *$p<0$ 01 Student's t test (n=4)

centrations from 0 1 ng/ml to 1 μg/ml significantly increased total β-adrenoceptors (by about 25%). When only surface β-adrenoceptors were measured (^{3}H-dihydroalprenolol binding inhibited by 1 μmol/l CGP 12177) the effect of corticosterone (0.5 μg/ml) was even more pronounced (70% increase over control value)

To obtain reasonable amounts of blood in the present investigation it was collected from the decapitated trunk of the rats However, decapitation is associated with a 10-fold increase in circulating norepinephrine and an 80-fold increase in circulating epinephrine [30] and the β-adrenoceptors on lymphocytes are therefore exposed to these high concentrations of catecholamines. An effect of this on surface receptors would be confounding in the interpretation of the present results on stress-induced receptor changes To exclude this possibility, surface β-adrenoceptors were compared on lymphocytes isolated from blood collected from an indwelling catheter (this procedure alters slightly circulating catecholamines [30] and from the decapitated trunk (Table 2) Specific ^{3}H-dihydroalprenolol binding to the surface β-adrenoceptors was similar in the two experimental groups and it was also the same in a third group in which isoproterenol (10 μmol/l final concentration) was added just after blood collection (Table 2, group C). This lack of difference is probably due to the experimental procedure (blood collected on ice, rapidly diluted and washed several times) that did not allow any catecholamine-receptor interaction (sufficient to induce receptor changes).

TABLE 2

EFFECT OF DIFFERENT BLOOD COLLECTION PROCEDURES ON LYMPHOCYTE SURFACE β-ADRENOCEPTORS

	Group A n=3	Group B n=3	Group C n=3
	Indwelling catheter	Decapitation	Decapitation + 10 μM isoproterenol
^{3}H-DHA (2 5 nM) specific binding	2 97 ± 0 81	2.94 ± 0.75	2 33 ± 0 74

Specific binding to surface receptors was measured as ^{3}H-dihydroalprenolol (^{3}H-DHA) inhibited by 1 μmol/l CGP 12177. Values are means ± SEM

DISCUSSION

Using intact lymphocytes as a model to study acute β-adrenoceptor response to stress, we observed a significant, transient receptor decrease in young rats after 30 min immobilization The most likely explanation of this is that it reflects an adaptive response (desensitization) to the increased catecholamine secretion induced by stress. In fact forced immobilization activates the sympatho-adrenal medullary system resulting in elevated plasma levels of catecholamines [6,26], and the concentration of β-adrenoceptors is reciprocally correlated with the ambient level of catecholamines [14]. According to this hypothesis the number of β-adrenoceptors was lowered in intact cells after 20–60 min *in vitro* exposure to β-adrenergic agonists [11, 15, 19, 24]. However overstimulation of β-adrenoceptors *in vivo*, either by increasing endogenous circulating catecholamines or by infusing β-adrenoceptor agonists, gave conflicting results β-Adrenoceptors were reduced in the rat pineal gland after 30 min isoproterenol infusion [23].

In man lymphocyte β-adrenoceptor number was unchanged [13,34] or raised [2] after increasing circulating catecholamines by postural changes or dynamic exercise. Intravenous infusion of adrenaline or isoprenaline to human subjects raised the number of β-adrenoceptors in membranes

from mononuclear leukocytes in 30 min, while continued infusion decreased the number by half in 4 hours [39] Similar biphasic changes have been found after acute exercise, but with a different time course (significant increase just after and decrease 25 min after exercise) [3] In contrast, during 10–60 min isoprenaline infusion Krall *et al* [24] found unchanged ^{3}H-dihydroalprenolol specific binding to intact lymphocytes

These reports, including the present findings, greatly differed in their methodological protocols, experimental design and, most important, in the degree of stimulation of β-adrenoceptor *in vivo* This may be important in explaining some of the above conflicting observations [2] The discrepancy between *in vitro* and *in vivo* results is noteworthy *In vitro* agonist-induced loss of receptors has been measured on intact cell systems while most of the *in vivo* studies measured receptors on homogenized membrane preparations It is likely that radioligand binding to intact or broken cells did not measure exactly the same receptor sites In fact a possible loss or inactivation of binding sites during preparation of cell particulate was suggested by the lower number of β-adrenoceptors and isoproterenol-stimulated cyclase activity found in broken cells [22] Therefore an effect of cell homogenization procedures on the agonist-induced receptor change, a rapidly reversible phenomenon [11,19], cannot be excluded All these considerations prompted us to use intact cells as providing more reliable information on dynamic changes of β-adrenergic receptors [31]

A recent paper [5] reported that the presence of exogenous (added *in vitro*) or endogenous (incompletely washed during membrane preparation) norepinephrine in the binding assay resulted in an apparent reduction of α_2 receptor number (Bmax) without any change in the K_D This effect, probably due to pseudo-irreversible binding of the agonist to the high affinity form of the receptor, was abolished when binding was determined in the presence of a high concentration of GTP or NaCl (or when the membranes were prepared in the presence of these two agents) The possibility that pseudo-irreversible binding of endogenous catecholamines (raised during stress) to β-adrenergic receptors accounted for the observed reduction in lymphocyte receptor number must be therefore considered However several reasons suggest that this is highly unlikely (1) In our experimental conditions the pseudo-irreversible binding of agonist to β-adrenergic receptors was not found In several experiments (in intact human lymphocytes, using ^{3}H-CGP 12177 as ligand) we found that isoproterenol affected the K_D, but not the Bmax (data not presented), (2) intact lymphocytes physiologically contain high concentrations of GTP and the binding in an intact cell system is comparable to that of membrane *in the presence* of GTP [22,29] Therefore pseudo-irreversible binding of the agonist is not to be expected, (3) The procedure used to isolate lymphocytes ensures almost complete removal of catecholamines, as shown by the similar receptor number in lymphocytes from blood containing widely different levels of agonists (Table 2)

The *in vitro* experiments showing the modulatory effect of isoproterenol (Fig 5) and corticosterone (Fig 7) on β-adrenoceptors raised several important issues related to the interpretation of data obtained from stress

In previous studies the effect of *in vitro* catecholamine treatment on intact cell systems was investigated using selective ligands such as ^{3}H-dihydroalprenolol (or other lipophilic ligand) and ^{3}H-CGP 12177 for measuring respectively total and surface β-adrenergic receptors Galant and Britt [15] reported that preincubation of human leukocytes with a high concentration of isoproterenol (100 μmol/l) induced a large loss (≈80%) of isoproterenol-stimulated adenylate cyclase and a less pronounced decrease (≈30%) of ^{3}H-dihydroalprenolol specific binding Using ^{3}H-CGP 12177 to measure surface β-adrenoceptors, a marked reduction (>50%) of receptor number was seen in intact cells exposed to 1–10 μmol/l isoproterenol [11, 19, 35] The present result confirms and extends these findings, by a new approach which gives a parallel estimate of total and surface β-adrenoceptors In fact in the same experiment ^{3}H-dihydroalprenolol was inhibited by propranolol (total receptors) or CGP 12177 (surface receptors)

Using this method, we found that high-concentration isoproterenol (100 μmol/l) pretreatment induced a slight decrease (−25%) of total β-adrenoceptors (Fig 5B) Surface receptors were drastically reduced even by 1 μmol/l isoproterenol (Fig 5A) This indicates a preferential role of surface receptors in agonist-induced desensitization on intact cells Therefore the reduction in ^{3}H-dihydroalprenolol binding after stress probably reflects adapative changes of surface β-adrenoceptors to the stress-induced increase of catecholamine

This was directly proved by the reduction of surface β-adrenergic receptor number (^{3}H-CGP 12177 binding) in restrained rats (Fig 3)

In accordance with previous results on human lymphocytes [7], ^{3}H-dihydroalprenolol specific binding to rat intact lymphocytes was significantly higher in the presence of corticosterone (Fig 7) Since this effect was seen after short exposure (15 min) of lymphocytes to corticosterone, the increase in β-adrenoceptors is probably explained by unmasking of criptic receptors (mediated by an effect of corticosterone on membrane fluidity) rather than receptor neosynthesis However, this point remains to be clarified

The efficacy of glucocorticoids in reversing β-adrenoceptor desensitization by agonists has been repeatedly described [21,33]. Therefore the elevation of corticosterone seen in our experiments could be important for the restoration of β-adrenoceptor basal values after the reduction at 30 min stress

The present study demonstrated that the receptor change induced by stress was modified in aged rats (Fig 2) The decrease in β-adrenoceptors in aged animals was less pronounced (−28%) and occurred much later (180 min) The release of catecholamines in response to immobilization stress was reportedly substantially similar in 3 and 28-month-old Fisher-344 rats [6] This makes it unlikely that the different receptor changes in young and aged animals are due to differences in catecholamine release induced by stress

An alternative explanation is that the retarded receptor response to stress is due to some age-dependent alteration of the intrinsic mechanisms of β-adrenoceptor regulation According to this hypothesis we found that the ability of isoproterenol (added *in vitro*) to reduce surface β-adrenoceptors was less pronounced in lymphocytes from aged rats (Fig 6) An altered adaptive receptor-response was suggested by existing evidence that β-adrenoceptors are less able to develop supersensitivity in the pineal gland of aged rats [17,42] However the receptor hyposensitivity induced by chronic desmethyl-imipramine was similar in young and aged rats [42] Thus the mechanisms of the altered receptor-regulation with aging appear to be complex and several aspects such as duration of stimulation (acute vs chronic), target organs (lymphocytes vs brain) and type of receptor response

(hypo-vs. hyper-sensitivity) need further investigation before any firm conclusion can be drawn.

To sum up, immobilization stress in young rats induced a complex series of short term events, including release of catecholamines and corticosterone and a decrease of β-adrenoceptors. The latter effect is probably due to the high plasma catecholamine levels while corticosterone could be important for receptor recovery from the desensitized to their basal state. This complex series of adaptive responses to stress seems to be impaired in aged animals.

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REFERENCES

1 Algeri, S , G Calderini, G Lomuscio, G Vantini, G Toffano and F Ponzio. Changes with age in rat central monoaminergic system responses to cold stress *Neurobiol Aging* **3**: 237–242, 1982
2 Brodde, O -E , A Daul, and N O'Hara β-Adrenoceptor changes in human lymphocytes, induced by dynamic exercise. *Naunyn Schmiedebergs Arch Pharmacol* **325**: 190–192, 1984
3 Butler, J , J G Kelly, K O'Malley and F Pidgeon β-Adrenoceptor adaptation to acute exercise *J Physiol* **344**: 113–117, 1983
4 Casale, T B , M. Halonen and M Kaliner Detection of beta-adrenergic receptors on rabbit mononuclear cells isolated free of significant contamination by other cell types *Life Sci* **33**: 971–977, 1983
5 Cheung, Y -D , D B Barnett and S R Nahorski. Interactions of endogenous and exogenous norepinephrine with alpha$_2$ adrenoceptor binding sites in rat cerebral cortex *Biochem Pharmacol* **33**: 1293–1298, 1984
6 Chiueh, C C , S M. Nespor and S I Rapoport Cardiovascular, sympathetic and adrenal cortical responsiveness of aged Fischer-344 rats to stress *Neurobiol Aging* **1**: 157–163, 1980
7 Cotecchia, S and A De Blasi Glucocorticoids increase β-adrenoceptors on human intact lymphocytes "in vitro " *Life Sci* **35**: 2359–2364, 1984
8. Davies, A O and R J Lefkowitz Corticosteroid-induced differential regulation of β-adrenergic receptors in circulating human polymorphonuclear leukocytes and mononuclear leukocytes *J Clin Endocrinol Metab* **51**: 599–605, 1980.
9 De Blasi, A and T Mennini Selective reduction of one class of dopamine receptor binding sites on the corpus striatum of aged rats *Brain Res* **242**: 361–364, 1982.
10 De Blasi, A , S Cotecchia and T. Mennini Selective changes of receptor binding in brain regions of aged rats *Life Sci* **31**: 335–340, 1982
11 De Blasi, A , S Cotecchia, M Fratelli and M Lipartiti Agonist-induced beta-adrenoceptor internalization on intact human mononuclear leukocytes effect of temperature of mononuclear leukocyte separation *J Lab Clin Med*, in press, 1986
12 De Souza, E B and G R Van Loon Stress-induced inhibition of the plasma corticosterone response to a subsequent stress in rats A nonadrenocorticotropin-mediated mechanism *Endocrinology* **110**: 23–33, 1982
13 Feldman, R D , L E Limbird, J Nadeau, G A FitzGerald, D Robertson and A J J Wood Dynamic regulation of leukocyte beta adrenergic receptor-agonist interactions by physiological changes in circulating catecholamines *J Clin Invest* **72**: 164–170, 1983
14 Fraser, J., J Nadeau, D Robertson and A. J J Wood Regulation of human leukocyte beta receptors by endogenous catecholamines. Relationship of leukocyte beta receptors density to the cardiac sensitivity to isoproterenol *J Clin Invest* **67**: 1777–1784, 1981
15 Galant, S P and S Britt Uncoupling of the beta-adrenergic receptor as a mechanism of in vitro neutrophil desensitization *J Lab Clin Med* **103**: 322–332, 1984
16 Garattini, S and A De Blasi. Brain receptors and aging in relation to drug effect In *Aging and Drug Therapy*, edited by C Barbagallo-Sangiorgi and A N Exton-Smith New York Raven Press, 1984, pp 9–22
17 Greenberg, L H and B Weiss β-Adrenergic receptor in aged rat brain Reduced number and capacity of pineal gland to develop supersensitivity *Science* **201**: 61–63, 1978.
18 Guillemin, R , G W Clayton, H. S. Lipscomb and J D Smith. Fluorometric measurement of rat plasma and adrenal corticosterone concentration *J Lab Clin Med* **53**: 830–832, 1959.
19 Hertel, C and M Staehelin Reappearance of β-adrenergic receptors after isoproterenol treatment in intact C6-cells *J Cell Biol* **97**: 1538–1543, 1983
20 Hoyer, D , E E. Reynolds and P B Molinoff Agonist-induced changes in the properties of beta-adrenergic receptors on intact S49 lymphoma cells *Mol Pharmacol* **25**: 209–218, 1984.
21 Hui, K K P , M E. Conolly and D P Tashkin Reversal of human lymphocyte β-adrenoceptor desensitization by glucocorticoids *Clin Pharmacol Ther* **32**: 566–571, 1982
22 Insel, P A. and L M Stoolman Radioligand binding to beta adrenergic receptors of intact cultured S49 cells *Mol Pharmacol* **14**: 549–561, 1978
23 Kebabian, J W , M Zatz, J A Romero and J Axelrod Rapid changes in rat pineal β-adrenergic receptor Alterations in l-[^{3}H]alprenolol binding and adenylate cyclase *Proc Natl Acad Sci USA* **72**: 3735–3739, 1975
24 Krall, J F , M Connelly and M L Tuck. Acute regulation of beta adrenergic catecholamine sensitivity in human lymphocytes *J Pharmacol Exp Ther* **214**: 554–560, 1980.
25 Krawietz, W and E Erdmann Specific and unspecific binding of [^{3}H](−)-dihydroalprenolol to cardiac tissue. *Biochem Pharmacol* **28**: 1283–1288, 1979
26 Kvetnansky, R , R McCarty, N B Thoa, C R Lake and I J Kopin Sympatho-adrenal responses of spontaneously hypertensive rats to immobilization stress *Am J Physiol* **236**: H457–H462, 1979
27 Lakatta, E G Age-related alterations in the cardiovascular response to adrenergic mediated stress *Fed Proc* **39**: 3173–3177, 1980

28 Oehler, J R , R B Herberman, D A Campbell, Jr and J Y Djeu Inhibition of rat mixed lymphocyte cultures by suppressor macrophages *Cell Immunol* **29:** 238–250, 1977
29 Pittman, R N and P B Molinoff Interaction of full and partial agonists with beta-adrenergic receptors on intact L6 muscle cells *Mol Pharmacol* **21:** 398–408, 1983
30 Popper, C W , C C Chiueh and I J Kopin Plasma catecholamine concentrations in unanesthetized rats during sleep, wakefulness, immobilization and after decapitation *J Pharmacol Exp Ther* **202:** 144–148, 1977
31 Porzig, H Are there differences in the β-receptor-adenylate cyclase systems of fragmented membranes and living cells? *Trends Pharmacol Sci* **3:** 75–78, 1982
32 Porzig, H , C Becker and H Reuter Competitive and non-competitive interactions between specific ligands and beta-adrenoceptors in living cardiac cells *Naunyn Schmiedebergs Arch Pharmacol* **321:** 89–99, 1982
33 Scarpace, P J and I B Abrass Desensitization of adenylate cyclase and down regulation of beta adrenergic receptors after in vivo administration of beta agonist *J Pharmacol Exp Ther* **223:** 327–331, 1982
34 Sowers, J R , M Connelly-Fittibghoff, M L Tuck and J F Krall Acute changes in noradrenaline levels do not alter lymphocyte β-adrenergic receptor concentrations in man *Cardiovasc Res* **17:** 184–188, 1983
35 Staehelin, M and P Simons Rapid and reversible disappearance of β-adrenergic cell surface receptors *EMBO J* **1:** 187–190, 1982
36 Staehelin, M , P Simmons, K Jaeggi and N Wigger CGP-12177 A hydrophilic β-adrenergic receptor radioligand reveals high affinity binding of agonists to intact cells *J Biol Chem* **258:** 3496–3502, 1983
37 Stone, E A and J E Platt Brain adrenergic receptors and resistance to stress *Brain Res* **237:** 405–414, 1982
38 Tanaka, M , Y Kohno, A Tsuda, R Nakagawa, Y Ida, K Iimori, Y Hoaki and N Nagasaki Differential effects of morphine on noradrenaline release in brain regions of stressed and non-stressed rats *Brain Res* **275:** 105–115, 1983
39 Tohmeh, J F and P E Cryer Biphasic adrenergic modulation of β-adrenergic receptors in man Agonist-induced early increment and late decrement in β-adrenergic receptor number *J Clin Invest* **65:** 836–840, 1980
40 Toews, M and J P Perkins Agonist-induced changes in β-adrenergic receptors on intact cells *J Biol Chem* **259:** 2227–2235, 1984
41 Watanabe, Y , R T Lai, H Higuchi, H Maeda and H Yoshida Beta-adrenoceptors of human lymphocytes Decrease in affinity of agonist for (−)^{3}H-dyhydroalprenolol binding sites by cytoplasmic fractions *Life Sci* **28:** 1399–1405, 1981
42 Weiss, B , L Greemberg and E Cantor Age-related alterations in the development of adrenergic denervation supersensitivity *Fed Proc* **38:** 1915–1921, 1979