# Stress-induced alteration in the lipolytic response to β-adrenoceptor agonists in rat white adipocytes

**Elisângela Farias-Silva, Dora Maria Grassi-Kassisse, Valéria Wolf-Nunes, and Regina Célia Spadari-Bratfisch[1]**

Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP-Brasil

**Abstract** **We analysed the sensitivity to β-adrenoceptor agonists in epididymal adipose cells from rats submitted to a stress protocol previously reported to induce alterations in sensitivity to catecholamines in cardiac tissue from rats. Food intake and body weight were lower, whereas adipocytes basal lipolysis was higher (control: 0.59 ± 0.04; stress: 1.00 ± 0.11, μmol glycerol/100 mg total lipids/100 min) in stressed compared to control rats. The responses to isoprenaline ($pD_2$ control: 7.46 ± 0.11; stress: 8.11 ± 0.17), adrenaline ($pD_2$ control: 5.78 ± 0.20; stress: 6.13 ± 0.18), and salbutamol ($pD_2$ control: 5.64 ± 0.28; stress: 5.92 ± 0.34) were sensitized, and the lipolytic responses to norepinephrine ($pD_2$ control: 6.98 ± 0.13; stress: 6.41 ± 0.12) and to BRL37344 ($pD_2$ control: 8.43 ± 0.19; stress: 7.54 ± 0.21) were desensitized. Responses to the higher concentration (100 μM) of isoprenaline (control: 1.80 ± 0.18; stress: 2.24 ± 0.10 μmol glycerol/100 mg total lipids/100 min), epinephrine (control: 1.64 ± 0.17; stress: 2.24 ± 0.14 μmol glycerol/100 mg total lipids/100 min), salbutamol (control: 0.65 ± 0.11; stress: 1.21 ± 0.41 μmol glycerol/100 mg total lipids/100 min), and d-butyryl-cAMP (control: 1.59 ± 0.17; stress: 2.72 ± 0.25) were significantly enhanced in adipocytes from stressed rats. $pD_2$ or maximum response to CGP12177 were not altered. Supersensitivity to isoprenaline was abolished by 50 nM ICI118,551 but was not modified by 100 nM metoprolol. However, subsensitivity to norepinephrine and to BRL37344 was abolished by 100 nM metoprolol. Our results suggest that in epididymal adipocytes from stressed rats there is a desensitization of the response to adrenoceptor agonists mediated by $β_1$-adrenoceptors together with a sensitization of the response mediated by $β_2$-adrenoceptors. $β_3$-adrenoceptors seem to be resistant to the stress effect.**—Farias-Silva, E., D. M. Grassi-Kassisse, V. Wolf-Nunes, and R. C. Spadari-Bratfisch. **Stress-induced alteration in the lipolytic response to β-adrenoceptor agonists in rat white adipocytes.** ***J. Lipid Res.*** **1999.** 40: **1719–1727.**

**Supplementary key words** adrenergic receptors • footshock stress • epididymal adipocytes • sensitivity

It is now well established that at least three β-adrenoceptor subtypes ($β_1$-, $β_2$-, and $β_3$-) coexist in the fat cells of the white adipose tissue of rat (1) or other mammalian species (2, 3). The β-adrenoceptors are positively coupled to adenylyl cyclase (4), so that an increase in cAMP levels causes stimulation of lipolysis after activation of cAMP-dependent protein kinase which phosphorylates the hormone-sensitive lipase (5). The coexistence of three receptor subtypes involved in the same biological effect suggests a complex regulation of adipose tissue responses to endogenous catecholamines during physiological processes involving lipomobilization (6).

The expression and function of each β-adrenoceptor subtype are independently regulated by homologous and heterologous agents which are able to cause desensitization or sensitization of the response mediated by each of the three adrenoceptor subtypes (7). Catecholamines and glucocorticoids have been reported to play a major role in this regulation process. These same hormones have been considered to be "stress hormones" (8). We have shown previously that sensitivity to catecholamines is modified in right atria from footshock stressed rats (9–15). The aim of the present study was to investigate whether the same stress protocol would induce any alteration in the lipolytic response to adrenoceptor agonists in adipocytes from rat epididymal adipose tissue.

## MATERIAL AND METHODS

### Animals

Male Wistar rats (*Rattus norvergicus*) weighing 200–350 g at the beginning of the experiments were used. The animals were housed in standard cages in a temperature-controlled room (22°C), with a 12 h light/12 h dark cycle with lights on at 6:30 am. Standard laboratory chow and tap water were available ad libitum.

Rat body weight was determined at the first and at the last day of the experiment. Daily chow consumed was also determined during the same period for control and stressed rats. During the experiments, the animals were cared for in accordance with the principles for the use of animals for research and education, fol-

[1] To whom correspondence should be addressed.

lowing Statement of Principles, which have been adopted by the FASEB Board.

### Stress protocol

Rats were individually submitted to three daily sessions of unsignaled inescapable footshock. The animals were placed in a plexiglas chamber (26 cm long × 21 cm wide × 26 cm high) provided with a grid floor consisting of stainless-steel rods (0.3 cm in diameter and spaced 1.0 cm apart). During the 30-min footshock sessions, which were held between 7:30 and 11:00 am, footshocks were delivered by a constant current, i.e., source controlled by a microprocessor-based instrument constructed at the Biomedical Engineering Centre of the Universidade Estadual de Campinas. Each rat received 120 footshocks. Current intensity was 1.0 mA and duration was 1.0 s at random intervals of 5–25 sec with a mean interval of 15 sec (16). After the end of the first and second footshock sessions, the animals were returned to their cages. After the third session rats were killed by a blow on the head and blood was collected for serum corticosterone analyses. All stress protocols have been reported to induce modifications in sensitivity to catecholamines in right atria isolated from male rats (9, 10) and female rats at diestrus (17).

### Adipocyte preparation and lipolysis measurements

The animals were killed by a blow to the back of the head and exsanguination. Epididymal white adipose tissue from male rats was rapidly dissected out. Lipolytic activity was analyzed on isolated fat cells obtained according to the method of Rodbell (18), with minor modifications. Krebs Ringer bicarbonate buffer containing bovine serum albumin (3%) and glucose (6 mm) (KRBA), adjusted to pH 7.4 with 1 m NaOH just before use, was utilized. After collagenase treatment (1 mg/ml), isolated fat cells were filtered through a nylon mesh, washed three times, and the packed cells were adjusted to a suitable dilution with KRBA buffer. The cells were incubated in plastic vials (1 ml of incubation medium) with gentle shaking in a water bath, at 37°C, with 10 μl pharmacological agents for 100 min (preincubation with antagonist for 40 min, plus incubation with agonist for 60 min). Pharmacological agents at suitable dilutions were added to the cell suspension just before the beginning of the assay. After incubation, the tubes were placed in an ice bath, and 200-μl aliquots of the infranatant medium were taken for enzymatic determination of glycerol (19), which was used as the index of fat cell lipolysis. Total lipid was evaluated gravimetrically after extraction (20). Concentration–response curves for agonists were constructed from experiments carried out in the presence and absence of the antagonist. Half-maximal effective drug concentration ($EC_{50}$) values were obtained and expressed as $pD_2$ values ($-\log EC_{50}$). For full agonists, the values were normalized by assuming the maximal effect of each agonist in the control groups to be 100%; for partial agonists the values were plotted as percentage of isoprenaline maximal effect in the control group.

### Serum corticosterone levels

Blood samples from control and stressed rats were centrifuged (room temperature, 10 min, 2300 *g*) and serum corticosterone levels were determined by RIA (ICN Pharmaceuticals).

### Drugs and chemicals

ATP, bovine serum albumin (fraction V, ), *Clostridium histolyticum* collagenase type II, epinephrine, glycerol kinase from *Candida micoderma*, glycerol phosphate dehydrogenase type I from rabbit muscle, (−)-isoproterenol, NAD, and (−)-norepinephrine were from Sigma Chemical Company (St. Louis, MO); BRL37344, ICI118,551, and salbutamol were from Tockris Cookson (St. Louis, MO); Da Rat Corticosterone kit was from ICN Pharmaceuticals, Inc (Orangeburg, NY).

### Statistical analysis

Data were analyzed statistically by one-way analysis of variance followed by the Fischer test or by Student's *t*-test for unpaired samples (21, 22). Differences were considered significant at $P < 0.05$.

## RESULTS

### Body weight and food intake

The mean body weight of control rats was 342.2 ± 9.7 g (n = 14) at the first day of experiments and 347.1 ± 6.3 g at the third day; the difference was not statistically significant (Student's *t*-test, $P > 0.05$). Mean body weight of rats from the stressed group was not different from control group at the first day, before footshock session (341.82 ± 5.2 g, n = 17). After the third footshock session, rat body weight was 332.2 ± 4.2 g; 9.6 ± 3.2 g lower than weight at the first day (Student's *t*-test, $P < 0.05$). Mean epididymal fat pad weight from that of the control rats was not different from that of stressed rats (2.56 ± 0.18 and. 2.99 ± 0.20, respectively).

Daily food intake of control rats was 34.4 ± 3.5 g (n = 17) whereas food intake of footshock-stressed rats was equal to 24.2 ± 0.7 g (n = 24; $P < 0.05$) during the 24 h after the first stress session and 27.3 ± 3.0 g (n = 24; $P > 0.05$) at 24 h after the second stress session. Rats were killed after the third footshock stress session.

Rats' serum corticosterone levels were higher after the third footshock session (61.40 ± 4.05 ng/ ml, n = 13) as compared to control rats (40.84 ± 6.52 ng/ml, n = 13).

### Lipolytic activity of β-agonists

To investigate the alteration of sensitivity in epididymal adipocytes from stressed rats, the functional effects of different β-adrenoceptor agonists and of d-butyryl-cAMP were examined. Adipocytes from rats submitted to footshock stress showed an increase in basal lipolysis (control: 0.59 ± 0.04; stress: 1.00 ± 0.11 μmol glycerol/100 mg total lipids/100 min). The maximal lipolysis stimulated by d-butyryl-cAMP (control: 1.59 ± 0.17, stress: 2.72 ± 0.25 μmol glycerol/100 mg total lipids/100 min), isoprenaline (control: 1.80 ± 0.18; stress: 2.24 ± 0.10 μmol glycerol/100 mg total lipids/100 min), epinephrine (control: 1.64 ± 0.17; stress: 2.24 ± 0.14 μmol glycerol/100 mg total lipids/100 min), and salbutamol (control: 0.65 ± 0.11; stress: 1.21 ± 0.41 μmol glycerol/100 mg total lipids/100 min) was also enhanced in adipocytes isolated from footshock-stressed rats. Maximal lipolysis stimulated by norepinephrine (control: 1.73 ± 0.35; stress: 2.32 ± 0.24 μmol glycerol/100 mg total lipids/100 min), BRL37344 (control: 2.19 ± 0.19 ; stress: 1.88 ± 0.20 μmol glycerol/100 mg total lipids/100 min), and CGP12177 (control: 0.69 ± 0.14; stress: 0.96 ± 0.29 μmol glycerol/100 mg total lipids/100 min) was not significantly modified after stress (**Fig. 1**).

**Table 1** shows the $pD_2$ values and maximal lipolytic effect of several compounds which stimulate lipolysis in adipocytes from control and footshock stressed rats. In adipocytes from control rats the potency rank for the agonists was BRL37344 > isoprenaline ⩾ norepinephrine > epinephrine. The maximum responses to these adrenocep-

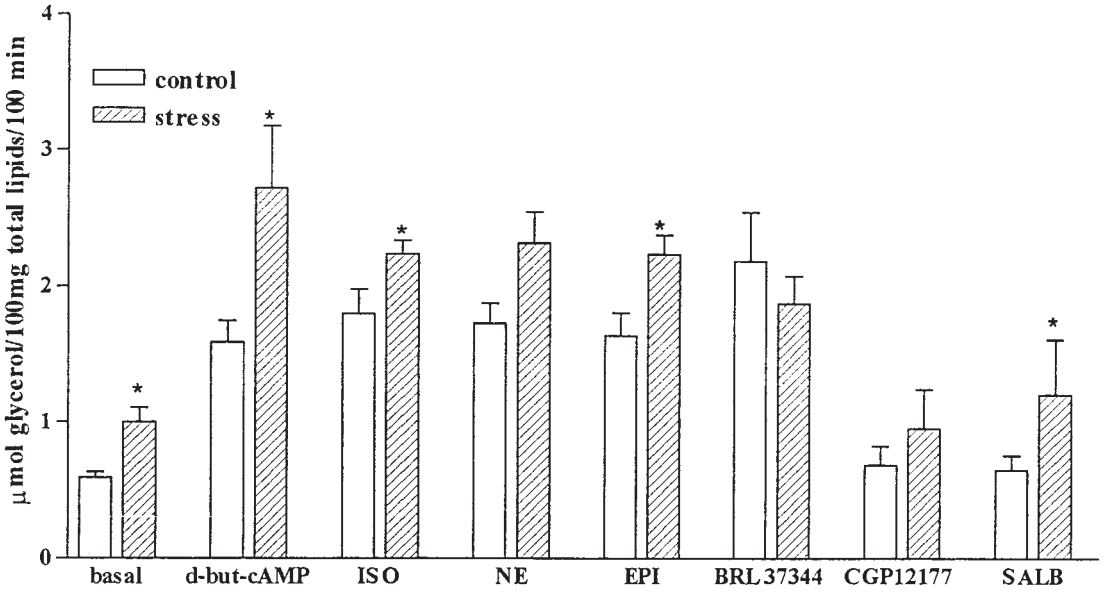


**Fig. 1.** Effects of stress on basal and maximal lipolysis in adipocytes isolated from control rats (white columns) or rats submitted to footshock stress (striped columns) after incubation for 100 min with 100 mm isoprenaline (ISO), norepinephrine (NE), epinephrine (EPI), BRL37344, CGP12177, salbutamol (SALB), or 1 mm d-butyryl-cAMP (d-but-cAMP). Basal lipolysis was determined in the absence of any agonist. Values are means ± SEM of experiments performed in duplicate. The numbers of experiments are given in Table 1. Student's *t*-test was used to compare the values between control and stressed groups. * Significantly different from control, at $P < 0.05$ (Student's *t*-test).

tor full agonists were not different from the response to d-butyryl-cAMP. Although the maximum response to adrenaline seems not to be obtained in the range of doses that we used, the lipolytic response obtained with the higher concentration (100 μm) was not different from the maximal response to BRL37344, isoprenaline, norepinephrine, or d-butyryl-cAMP, both in control or footshock-stressed rats. CGP12177 behaved as a partial agonist as the maximum response was significantly lower than the maximum response to isoprenaline or to d-butyryl-cAMP. Salbutamol maximum response was not reached by the higher dose that we used. However, there was a significant shift of the concentration response curve to the left in adipocytes from stressed rats compared to control. Whereas in adipocytes from control rats the response to the higher dose of salbutamol was only 28% and 36% of the maximal responses to BRL37344 and isoprenaline, respectively, in adipocytes from stressed rats the response to the dose of 100 μm of salbutamol was 64% and 54%, respectively, of the maximal responses to the cited β-adrenergic agonists. Moreover, in adipocytes from stressed rats, $pD_2$ value of isoprenaline and "apparent" $pD_2$ value of epinephrine were enhanced (4.5- and 2.2-fold, respectively, $P < 0.05$) with a shift to the left in concentration–response curves (**Fig. 2a, 2c**). On the other hand, $pD_2$ values of norepinephrine and BRL37344 were lower in adipocytes from stressed rats compared to control (3.7- and 7.8-fold, respectively, $P < 0.05$) and the concentration–response curves to both agonists were shifted to the right (Fig. 2b, 2d). As a consequence, in adipocytes from stressed rats, the order of relative potencies was modified: isoprenaline > BRL37344 > norepinephrine ⩾ epinephrine. The $pD_2$

TABLE 1. Lipolytic potency and maximal lipolytic responsiveness of adipocytes to different compounds

| | Control | | | Stress | | |
|---|---|---|---|---|---|---|
| Compounds | $pD_2$ | $E_{max}$ | n | $pD_2$ | $E_{max}$ | n |
| BRL 37344 | $8.43 \pm 0.19^{g}$ | $2.19 \pm 0.19^{d}$ | 5 | $7.54 \pm 0.21^{a}$ | $1.88 \pm 0.20^{d,f}$ | 3 |
| Isoprenaline | $7.46 \pm 0.11^{a}$ | $1.80 \pm 0.18^{d}$ | 5 | $8.11 \pm 0.17^{g}$ | $2.24 \pm 0.10^{f}$ | 3 |
| Norepinephrine | $6.98 \pm 0.13^{a}$ | $1.73 \pm 0.35^{d,f}$ | 6 | $6.41 \pm 0.12^{c}$ | $2.32 \pm 0.24^{f}$ | 3 |
| Epinephrine | $5.78 \pm 0.20^{b}$ | $1.64 \pm 0.17^{d}$ | 6 | $6.13 \pm 0.18^{c}$ | $2.24 \pm 0.14^{f}$ | 4 |
| CGP 12177 | $6.30 \pm 0.50^{b,c}$ | $0.69 \pm 0.14^{e}$ | 4 | $5.93 \pm 0.10^{b}$ | $0.96 \pm 0.29^{e}$ | 5 |
| Salbutamol | $5.64 \pm 0.28^{b}$ | $0.65 \pm 0.11^{e}$ | 3 | $5.92 \pm 0.34^{b,c}$ | $1.21 \pm 0.41^{f}$ | 3 |
| d-Butyryl cAMP | | $1.59 \pm 0.17^{d}$ | 5 | | $2.72 \pm 0.25^{f}$ | 3 |

The values are means ± SEM of the number of experiments (n) performed in duplicate. The potencies of the lipolytic agents were evaluated by their $EC_{50}$, which corresponds to the concentration of agonists inducing 50% of maximal lipolysis, expressed as $pD_2$ ($-$ log $EC_{50}$). $E_{max}$ is the maximal responsiveness minus basal lipolysis (control: 0.59 ± 0.04 and stress: 1.00 ± 0.11) and is expressed as μmol glycerol / 100 mg total lipids / 100 min. Statistical analysis was performed using ANOVA plus Fischer's test; control and stressed groups with the same agonist were compared by Student's *t*-test. Values followed by different letters are significantly different ($P < 0.05$).

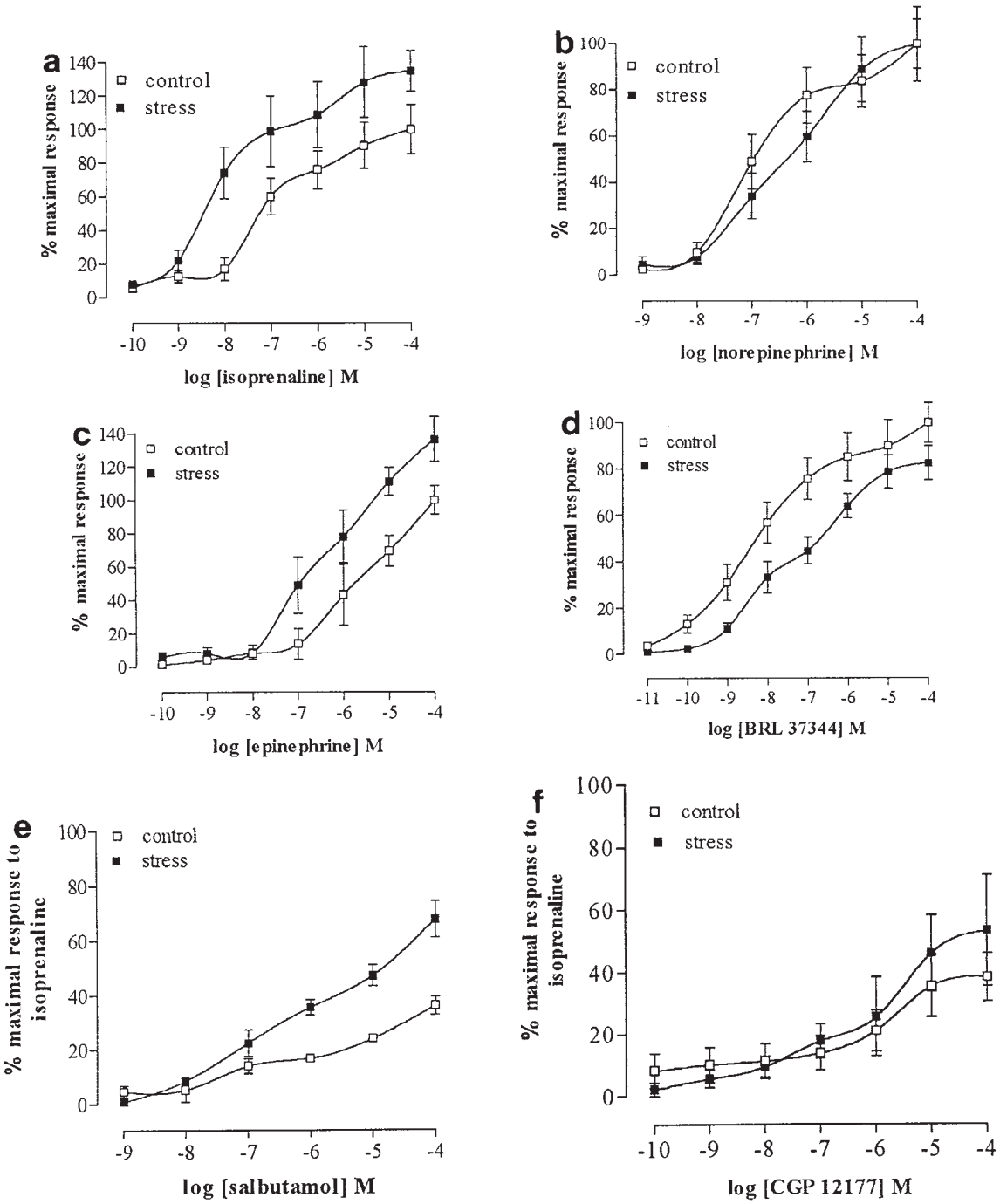


**Fig. 2.** Concentration-response curves for stimulation of glycerol release from white adipocytes of control (□) or footshock-stressed rats (■) elicited by isoprenaline (a), norepinephrine (b), epinephrine (c), BRL37344 (d), salbutamol (e), and CGP12177 (f). For isoprenaline, norepinephrine, epinephrine, and BRL37344, the values were normalized by assuming the maximal effect of the agonist in the control groups to be 100%; for salbutamol and CGP12117, the values were plotted as percentage of isoprenaline maximal effect in the control group. Data are means and vertical bars represent the SEM. The number of experiments is given in Table 1.

value for the partial agonist CGP12177 was not significantly altered after stress (Table 1, Fig. 2d, 2e).

### Effect of β-adrenoceptor antagonists

The sensitivity of adipocytes to isoprenaline was analyzed in the presence of 100 nm metoprolol (a $\beta_1$-adrenoceptor antagonist) or 50 nm ICI118,551 (a $\beta_2$-adrenoceptor antagonist). Basal lipolysis was increased in the presence of metoprolol in adipocytes from control (1.26 ± 0.21 μmol glycerol/100 mg total lipids/100 min) but was not modified in adipocytes from stressed rats (1.16 ± 0.21 μmol glycerol/100 mg total lipids/100 min). The maximal response to isoprenaline was not altered in the presence of any of the β-adrenoceptor antagonists (data not shown).

In adipocytes from control and stressed rats, concentration–response curves to isoprenaline obtained in

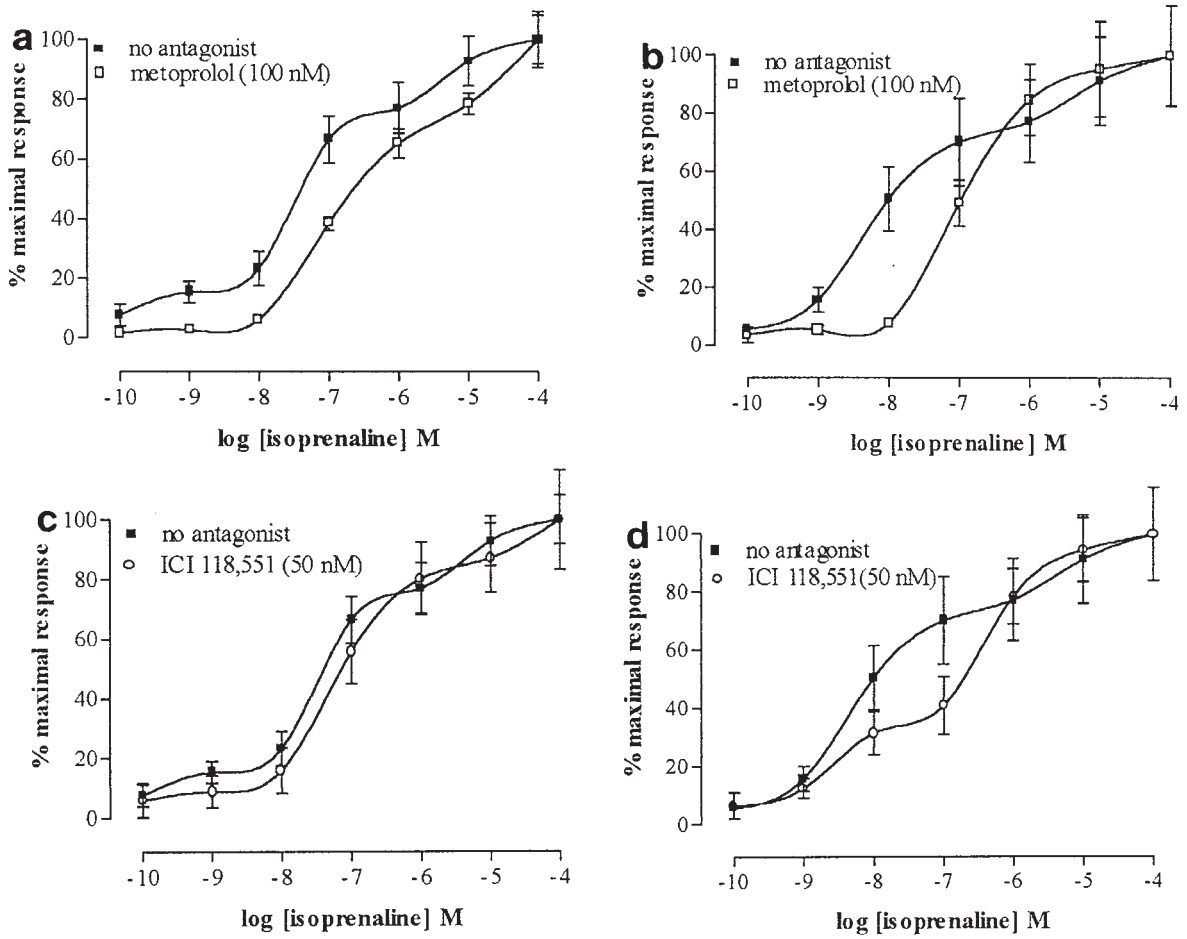


**Fig. 3.** Concentration–response curves for stimulation of glycerol release from white adipocytes isolated from control (a, c) or footshock-stressed rats (b, d) elicited by isoprenaline, in the absence (■) or in the presence of 100 nm metoprolol (□) or 50 nm ICI118,551 (○). Values were normalized by assuming the maximal effect of isoprenaline in each group to be 100%. Vertical bars represent the SEM. The number of experiments is given in Table 2.

the presence of metoprolol were significantly shifted to the right (13- and 22-fold, respectively; **Fig. 3,** panels **a, b**). The sensitivity of adipose cells from stressed rats remained higher than control (**Table 2**).

ICI118,551 (50 nm) had no significant effect on the concentration–response curve to isoprenaline obtained for adipocytes from control rats (Table 2). However, this

TABLE 2. Comparative antagonistic effects of 100 nm metoprolol and 50 nm ICI118,551 on the response to isoprenaline of rat white adipocytes

| | Control | | Stress | |
|---|---|---|---|---|
| | $pD_2$ | n | $pD_2$ | n |
| Isoprenaline | $7.45 \pm 0.05^{a}$ | 9 | $8.23 \pm 0.11^{e}$ | 6 |
| Isoprenaline + metoprolol | $6.33 \pm 0.03^{b}$ | 5 | $6.89 \pm 0.16^{d}$ | 5 |
| Isoprenaline + ICI118,551 | $7.04 \pm 0.26^{a,d}$ | 4 | $6.47 \pm 0.35^{b,d}$ | 5 |

Values are means ± SEM of the number of experiments (n) performed in duplicate. The potencies of isoprenaline were evaluated by $EC_{50}$, which is the concentration of the agonist inducing 50% of maximal lipolysis, expressed as $pD_2 = -\log EC_{50}$. Statistical analysis was performed by ANOVA plus Fischer's test. Values followed by different letters are significantly different ($P < 0.05$).

$\beta_2$-adrenoceptor antagonist induced a 58-fold shift to the right, at the $pD_2$ level ($P < 0.05$) in the concentration–response curve to isoprenaline in adipocytes from stressed rats. Moreover, in the presence of ICI118,551, adipocytes from stressed rats were subsensitive to isoprenaline compared to adipocytes from control rats ($P < 0.05$; Table 2; Fig. 3, panels c, d).

Metoprolol (100 nm) induced a shift to the right in the concentration–response curve to norepinephrine in adipocytes from control (13-fold at $pD_2$ level, $P < 0.05$) and stressed rats (4.7-fold at $pD_2$, $P < 0.05$) (**Fig. 4**) and a 6.5-fold shift in the concentration–response curve to BRL37344 in adipocytes from control rats ($P < 0.05$; **Fig. 5**). In the presence of metoprolol, sensitivity to norepinephrine in adipocytes from stressed rats was not different from control ($P < 0.05$).

Figure 5 shows that the concentration–response curve to BRL37344 was shifted to the right about 56-fold, at the $pD_2$ level ($P < 0.05$) in adipocytes from control rats, whereas in adipocytes from stressed rats which were subsensitive to BRL37344, metoprolol shifted the concentration–response curve to the right by about 6.5-fold ($P < 0.05$).

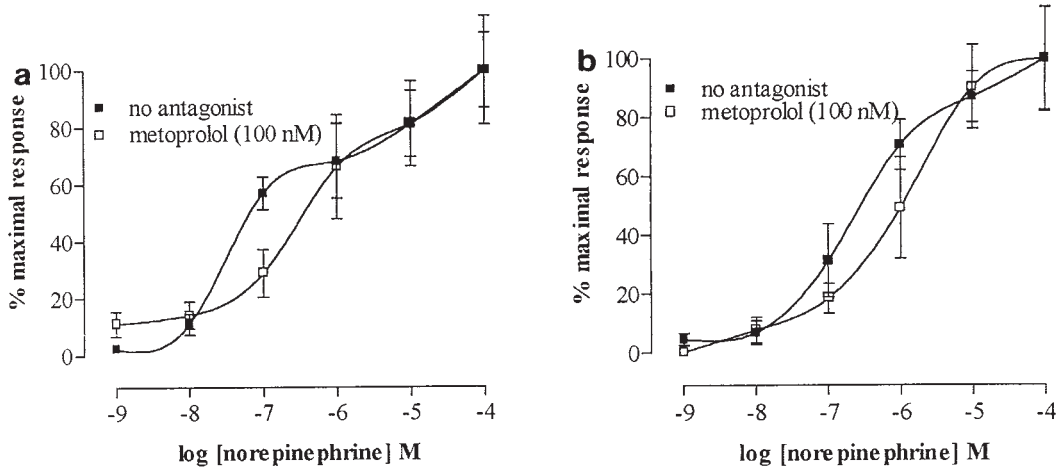


**Fig. 4.** Concentration–response curves for stimulation of glycerol release from white adipocytes isolated from control (a) or footshock-stressed rats (b) elicited by norepinephrine, in the absence (■) or in the presence of 100 nm metoprolol (□). Values were normalized by assuming the maximal effect of the isoprenaline in each group to be 100%. Vertical bars represent the SEM. The number of experiments is given in Table 3.

In the presence of 100 nm metoprolol stress-induced subsensitivity to BRL37344 was abolished.

## DISCUSSION

Our results have shown that rats submitted to three daily footshock sessions presented a significant body weight loss as compared to control animals at the same period, together with a decrease in the food intake and increased epididymal white adipocytes basal rate of lipolysis.

In rat white adipocytes, the $\beta_2$-subtype represents a very small population of β-adrenoceptors and under physiological conditions, the $\beta_2$-subtype is probably not implicated in the stimulation of lipolysis. The overall lipolysis is essentially dependent on $\beta_1$- and $\beta_3$-receptors and mainly driven by the $\beta_3$-subtype. Germack et al. (23) proposed the following rank order of expression and capacity to induce lipolysis as $\beta_3 >> \beta_1 > \beta_2$ in this tissue.

In the present study, the functional stimulation of lipolysis in adipocytes from control rats exhibited a rank order of agonist potencies (BRL 37344 >> isoprenaline ⩾ norepinephrine > epinephrine) which is in accordance with that previously described for rat and garden dormouse white adipocytes (23, 24). However, adipocytes from footshock-stressed rats present an increased sensitivity and maximum response to isoprenaline and epinephrine, together with subsensitivity to norepinephrine and BRL37344. Moreover, we have observed an increase in the maximal lipolytic effect stimulated by salbutamol but no alteration of the response to CGP12177. As a consequence, in adipocytes from stressed rats, the rank order of agonist potencies was modified as follows: isoprenaline > BRL37344 > norepinephrine ⩾ epinephrine.

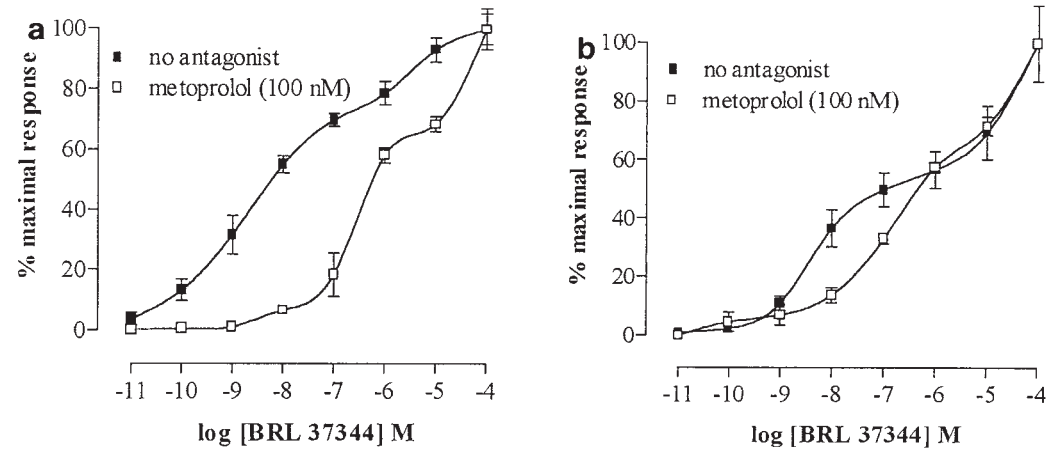


**Fig. 5.** Concentration–response curves for stimulation of glycerol release from white adipocytes isolated from control (a) or footshock-stressed rats (b) elicited by BRL37344, in the absence (■) or in the presence of 100 nm metoprolol (□). Values were normalized by assuming the maximal effect of the isoprenaline in each group to be 100%. Vertical bars represent the SEM. The number of experiments is given in Table 4.

Isoprenaline and epinephrine do not discriminate between $\beta_1$-, $\beta_2$-, and $\beta_3$-adrenoceptors (25) and norepinephrine exhibits low affinity for the $\beta_2$-subtype, while BRL37344 preferentially activates $\beta_3$-adrenoceptors, but might also activate $\beta_1$-adrenoceptors at high concentrations (26). Thus our results seem to indicate that in adipocytes from stressed rats there is an enhancement of the role played by $\beta_2$-adrenoceptors in mediating the lipolytic effect of catecholamines whereas the response mediated by $\beta_1$-adrenoceptor subtypes is desensitized. As the response to BRL37344 is also decreased, it might be suggested that $\beta_3$-adrenoceptor subtype has also been desensitized in adipocytes from stressed rats. However, the shift to the right observed in the concentration–response curve to BRL37344 is more pronounced at higher concentrations of norepinephrine (Fig. 2d), giving a biphasic pattern to the concentration–response curve and indicating that the response mediated by $\beta_1$-adrenoceptor rather than by the $\beta_3$-adrenoceptor has been desensitized.

Additional support for our hypothesis comes from the effect of antagonists on the responses to the agonists. Metoprolol, a $\beta_1$-adrenoceptor antagonist which also blocks $\beta_3$-adrenoceptors (27), shifted to the right the concentration–response curves to isoprenaline, norepinephrine, and BRL37344 in adipocytes from control and stressed rats. In the presence of metoprolol, adipocytes from stressed rats remained supersensitive to isoprenaline but the subsensitivity to norepinephrine and BRL37344 was abolished. The $pK_B$ value for metoprolol in adipocytes from control rats estimated from data in **Table 3** is 8.7. This indicates that the effect of BRL37344 was mostly via $\beta_1$-adrenoceptor. Indeed, reported $pA_2$ values for metoprolol acting at $\beta_1$-adrenoceptor are lower than 8.7 (7.6 in right atrium) (28) and more in line with the $pK_B$ of 8.1 that can be calculated for the antagonism of norepinephrine effect (**Table 4**). Metoprolol $pA_2$ values of 5.0 to 7.5 have been reported by others (29, 30) but it is most likely that the lower end of the range of these values reflects a $pK_B$ for $\beta_3$-adrenoceptors and the upper end of the range reflects a $pK_B$ for $\beta_1$-adrenoceptor. At the concentration of 100 nm, in which metoprolol blocks only $\beta_1$-adrenoceptors, the shift to the right observed in the concentration–response curve to BRL37344 in the presence of metoprolol might be due to a $\beta_1$-adrenoceptor-mediated effect of metoprolol.

CGP12177 has never been shown to stimulate $\beta_1$- or $\beta_2$-adrenoceptors in tissues and has been considered as a partial agonist of $\beta_3$-adrenoceptors (24). Moreover, some authors argue that CGP12177 acts via a putative $\beta_4$-adrenoceptor subtype, which is proposed to be present in white adipocytes from rats and the hearts of several animal species (31–33). The response to CGP12177 has not been altered in adipocytes from stressed rats which supports the argument that $\beta_3$-adrenoceptor and putative $\beta_4$-adrenoceptors, if present, are resistant to the stress effect.

ICI118,551, a selective $\beta_2$-adrenoceptor antagonist at the concentration used here (34) did not induce any shift in the concentration–response curves to isoprenaline in adipocytes from control rats, which is in agreement with the minor role, if any, of the $\beta_2$-adrenoceptor subtype in these cells. However, in adipose cells from stressed rats, the decrease in isoprenaline potency induced by ICI118,551 abolished the supersensitivity to the agonist. The concentration–response curve to isoprenaline in the presence of ICI118,551 was clearly biphasic with the blocking effect of the antagonist occurring at agonist concentrations varying between 10 nm and 1 μm. In the same tissue, there was an increase in the response to the higher concentration of salbutamol, a partial agonist with an affinity for the $\beta_2$-subtype around 50-fold higher than for $\beta_3$- and 15-fold higher than for the $\beta_1$-adrenoceptor subtype (34), indicating that the supersensitivity to isoprenaline might be due to an increase in $\beta_2$-adrenoceptor number.

Although we have not investigated the mechanisms underlying these stress-induced alterations in sensitivity to β-adrenoceptor agonists, our results suggest that in adipocytes from stressed rats there is a desensitization of the response mediated by $\beta_1$-adrenoceptors together with a sensitization of the response mediated by $\beta_2$-adrenoceptors. Similar results have been obtained for cardiac tissue isolated from rats submitted to the same stressor agent (17, 35).

Desensitization of the response to β-adrenergic agonists has been previously shown in rat adipocytes after chronic norepinephrine infusion (36, 37) or infusion of a $\beta_3$-agonist, CL 316 243 (38). Unelius et al. (39) demonstrated that prolonged exposure of hamsters to cold led to desensitization of $\beta_3$-adrenoceptors in brown adipocytes. Down-regulation of the $\beta_3$-adrenoceptor subtype has been shown upon exposure of adipose cells to glucocorticoids (40) and it has been proposed that glucocorticoids exert a differential regulation of the β-adrenoceptors in 3T3-

TABLE 3. Antagonism of the lipolytic effect of BRL37344 by 100 nm metoprolol in the white adipocytes from rats

| | Control | | Stress | |
|---|---|---|---|---|
| | $pD_2$ | n | $pD_2$ | n |
| BRL37344 | $8.29 \pm 0.25^a$ | 6 | $7.01 \pm 0.30^c$ | 6 |
| BRL37344 + metoprolol | $6.54 \pm 0.34^{b,c}$ | 4 | $6.20 \pm 0.26^b$ | 3 |

Values are means ± SEM of the number of experiments (n) performed in duplicate. The potencies of BRL37344 was evaluated by $EC_{50}$, which is the concentration of the agonist inducing 50% of maximal lipolysis, expressed as $pD_2 = -\log EC_{50}$. Statistical analysis was performed by ANOVA test plus Fischer's test. Values followed by different letters are significantly different ($P < 0.05$).

TABLE 4. Antagonism of the lipolytic effect of norepinephrine by 100 nm metoprolol in white adipocytes from rats

| | Control | | Stress | |
|---|---|---|---|---|
| | $pD_2$ | n | $pD_2$ | n |
| Norepinephrine | $7.29 \pm 0.15^a$ | 6 | $6.63 \pm 0.12^b$ | 5 |
| Norepinephrine + metoprolol | $6.18 \pm 0.23^{b,c}$ | 6 | $5.96 \pm 0.19^c$ | 5 |

Values are means ± SEM of the number of experiments (n) performed in duplicate. The potencies of norepinephrine were evaluated by $EC_{50}$, which is the concentration of the agonist inducing 50% of maximal lipolysis, expressed as $pD_2 = -\log EC_{50}$. Statistical analysis was performed by ANOVA plus Fischer's test. Values followed by different letters are significantly different ($P < 0.05$).

F442A cells at a transcriptional level. While these compounds enhance $\beta_2$-adrenoceptor expression, they strongly repress expression of $\beta_1$- and $\beta_3$-adrenoceptors (34, 40, 41). Rats presented a 50% increase in seric corticosterone level after the third footshock session as compared to control animals.

We have previously demonstrated that right atria isolated from rats submitted to three sessions of footshock stress present supersensitivity to isoprenaline and epinephrine as well as subsensitivity to norepinephrine (10). If the increase in serum corticosterone level is prevented by adrenalectomy or by treatment with metirapone, stress-induced alterations in cardiac sensitivity to catecholamines are not observed (14). Moreover, treatment of animals with RU38486, an antagonist of glucocorticoid receptors, also prevented the effects of stressor sensitivity to catecholamines in right atria from footshock-stressed rats (11).

The results presented here suggest that the stress-induced alterations in sensitivity of tissues to catecholamines are similar in cardiac and in adipose tissue and that a decrease in the $\beta_1$-adrenoceptor-mediated response might be accompanied by an increase in $\beta_2$-adrenoceptor-mediated response. If the switch in the β-adrenoceptor subtype mediating the effect of catecholamines on adipocytes is towards a $\beta_2$-subtype, this leads to a corresponding switch in the role played by noradrenaline, the sympathetic neurotransmitter, and adrenaline, the adrenal gland medullary hormone, in the control of lipolysis and might be related to the pathophysiological conditions of adipose tissue associated with stress.

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