Role of $\beta_1$- and $\beta_3$-adrenoceptors in the regulation of lipolysis and thermogenesis in rat brown adipocytes

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Atgié, Claude, François D'Allaire, and Ludwik J. Bukowiecki. Role of $\beta_1$- and $\beta_3$-adrenoceptors in the regulation of lipolysis and thermogenesis in rat brown adipocytes. Am. J. Physiol. 273 (Cell Physiol. 42):C1136–C1142, 1997.—To evaluate the physiological functions of $\beta_1$, $\beta_2$, and $\beta_3$-adrenoceptors (ARs) in brown adipose tissue, the lipolytic and respiratory effects of various adrenergic agonists and antagonists were studied in rat brown adipocytes. The $\beta$-agonists stimulated both lipolysis and respiration (8–10 times above basal levels), with the following order of potency (concentration eliciting 50% of maximum response): CL-316243 ($\beta_3$ >> BRL-37344 ($\beta_3$ >> isoproterenol (mainly $\beta_1$/$\beta_2$) >> norepinephrine (NE; mainly $\beta_1$/$\beta_3$) >> epinephrine (mainly $\beta_2$) >> dobutamine ($\beta_1$) >> procaterol ($\beta_2$). Schild plot coefficients of competitive inhibition experiments using ICI-113055 ($\beta_2$ antagonist) revealed that more than one type of receptor mediates NE action. It is concluded from our results that 1) NE, at low plasma levels (1–25 nM), stimulates lipolysis and respiration mainly through $\beta_2$-ARs, 2) NE at higher levels, stimulates lipolysis and respiration via both $\beta_1$- and $\beta_3$-ARs, 3) $\beta_2$-ARs play only a minor role, and 4) $\beta_3$-ARs may represent the physiological receptors for the high NE concentrations in the synaptic cleft, where the high-affinity $\beta_3$-ARs are presumably desensitized. It is also suggested that lipolysis represents the flux-generating step regulating mitochondrial respiration.

Brown adipose tissue; brown fat; respiration; $\beta_3$-adrenoceptors; norepinephrine; epinephrine; sympathetic nervous system.

In recent years, atypical or $\beta_3$-adrenoceptors (ARs) have been identified in a variety of tissues, and particularly in brown adipose tissue (BAT) (2, 3, 29). It has been well established that $\beta_1$, $\beta_2$, and $\beta_3$-ARs coexist in BAT and that they are all positively coupled to the adenyl cyclase system (7, 17, 23, 24). However, the relative contribution of the three receptor subtypes in mediating the metabolic effects of NE on BAT, remains to be defined. It is also known that ARs play only a minor role in mediating BAT metabolic functions (lipolysis, thermogenesis, and growth), at least in the rat (10, 20). It is known that NE binds a small number of $\beta_1$/$\beta_2$-ARs ($\sim 10^5$/cell) with high affinity (nanomolar level) and a large number of $\beta_3$-ARs ($\sim 10^6$/cell) with low affinity (micromolar level) (17, 33). In vivo, the concentration of circulating NE is very low (1–25 nM) (18, 19), and it is likely that at such low levels NE mainly binds the high-affinity receptors (17, 30). However, in contrast to epinephrine, NE is a neurohormone, and its concentration in the synaptic cleft may be sufficiently high to locally affect the low-affinity $\beta_3$-ARs. Furthermore, the contribution of the three $\beta$-ARs to the regulation of the various metabolic processes occurring in adipocytes (lipolysis, lipogenesis, thermogenesis, cell proliferation, differentiation, and so forth) may vary under different physiological conditions (cold acclimation, age of the animals, diet composition, and so on) (26).

The principal goal of the present studies was to examine the relative participation of the three $\beta$-receptor subtypes in the control of lipolysis and respiration by NE in isolated rat brown adipocytes. For this purpose, we compared the effects of NE (a mixed agonist) on lipolysis and respiration with those of various selective agonists. In addition, the effects of selective $\beta$-antagonists on NE-stimulated adipocytes were also investigated. Furthermore, parallel measurements of the effects of selective adrenergic agents on lipolysis and respiration enabled us to test the hypothesis that the rate of respiration in brown adipocytes is essentially controlled by the rate of lipolysis, i.e., that both phenomena are tightly coupled.

Results from these studies indicate that NE, at concentrations usually found in the circulation (1–25 nM), controls both lipolysis and respiration mainly via $\beta_2$-ARs, whereas, at much higher levels presumably occurring in the synaptic cleft after sympathetic stimulation (by cold exposure, diet, stress, and so forth), NE regulates these metabolic processes via both $\beta_1$- and $\beta_3$-adrenergic pathways.

METHODS

Brown adipocyte isolation. Female Sprague-Dawley rats weighing 250–300 g were kept at 27°C for at least 2 wk with a photoperiod of 12:12 light-dark and fed Purina Chow ad libitum. Brown adipocytes were isolated, essentially as previously described (9, 10, 17), from pooled interscapular BAT from 2–3 rats. In brief, cleaned pieces of tissue (0.9 g) were incubated for 15 min at 37°C in 2.5 ml of Krebs-Ringer-bicarbonate buffer containing 2.7 mM glucose, 1% albumin, and 20 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (KRB buffer; final pH 7.4), in the presence of collagenase (10 mg/ml) under an atmosphere of 95% O2/5% CO2. At the end of the digestion period, the cells were filtered through a nylon filter (500 µm), diluted in 10 ml of KRB buffer, and centrifuged (80 g) at room temperature for 5 min. The floating cells were filtered again through a filter (200 µm) and centrifuged following the same procedure. Finally, the isolated adipocytes were washed twice with 3 ml of KRB buffer and counted in a hemocytometer after trypan blue staining.

Oxygen consumption measurements. Oxygen uptake of brown adipocyte suspensions (1 × 10⁵ cells/ml in KRB buffer containing 4% albumin) was measured polarographically at 37°C in a water-jacketed Perspex chamber equipped with a Clark-style oxygen electrode, as previously described (9, 10).

Determination of the lipolytic rates. For the determination of lipolytic rates, the washed adipocytes were first diluted to a
concentration of 2-3 × 10^6 cells/ml in KRB buffer and then preincubated for 15 min at 37°C with gentle shaking (40 cycles/min) (9, 10). At the end of the preincubation period, the cells were washed twice with freshly bubbled buffer at 37°C. The adipocytes were then diluted at a concentration of 1.5 × 10^6 cells/ml in KRB and incubated for 45 min at 37°C. The lipolytic rates were estimated by measuring extracellular glycerol release, as previously described (9, 10).

Drugs and chemicals. (−)-NE bitartrate, (−)-epinephrine bitartrate, bovine serum albumin (fraction V), and collagenase (type II) were obtained from Sigma (St. Louis, MO). (−)-Propranolol, (−)-isoproterenol, and (±) dobutamine hydrochloride were purchased from RBI Biochemicals (Natick, MA). CL-316243 [disodium (R,R)-5-[2-[2-(3-chlorophenyl)-2-hydroxyethyl]-aminopoly]]-1,3-benzodioxole-2,2-dicarboxylate and BRL-37344 [4-[2-hydroxy-(3-chloro-phenyl)ethyl]-amino]-phenoxyacetate were kindly provided by Dr. T. H. Claus (American Cyanamid, Lederle Laboratories, Pearl River, NY) (8) and by Dr. M. A. Cawthorne (SmithKline-Beecham Pharmaceuticals, Epsom, UK), respectively. The following compounds were provided as generous gifts: ICI-89406 [1-(2-cyanophenox)-3-[β-(3-phenylureido) ethylamino]-2-propanol] from ICI Pharmaceuticals (Mississauga, ON, Canada) and CGP-20712A [(+)-2-(3-carbamoyl-4-trifluormethyl-2-imidazo-lyl)-phenoxy]-2-propanol methanesulphonate from Ciba-Geigy (Mississauga, ON, Canada). Procteral [OPC-2009; 5-[1-hydroxy-2-isopropylaminobuty]-8-hydroxocarbostyril hydrochloride hemihydrate] was kindly provided by Otsuka Pharmaceuticals (Tokushima, Japan).

RESULTS

Effects of NE and CL-316243 on the kinetics of brown adipocyte respiration. The goal of the experiment described in Fig. 1 was to compare the respiratory effects of NE, the physiological effector of thermogenesis, with those induced by CL-316243, a new agonist that has a high affinity and selectivity for β3-ARs (8). As previously reported, NE (100 nM) maximally stimulated brown adipocyte oxygen consumption, 10 times above basal values, within 3 min (Refs. 9, 10; see also Fig. 1).

These effects were entirely mimicked by CL-316243, added at a 10 times lower concentration than NE (10 nM). When maximal respiration had stabilized for a few minutes, propranolol (a β-agonist with a high affinity for β1/β2-ARs and a much lower affinity for β3-ARs) was injected in the respiratory chamber. In <3 min, propranolol (1 µM) inhibited 80–90% of the respiration stimulated by 10 times lower concentrations of NE (100 nM). However, under the same conditions, propranolol (1 µM) failed to affect respiration stimulated by 100 times lower concentrations of CL-316243 (10 nM). A 1,000 times higher concentration of propranolol (10 µM) still failed to affect CL-316243 (10 nM)-stimulated respiration, whereas it totally inhibited NE (100 nM)-induced respiration. Propranolol concentration had to be increased to 100 µM to observe a significant inhibition of CL-316243-induced respiration. Similar results were observed using other β3-agonists such as BRL-37344 (5). The observation that CL-316243-stimulated respiration is resistant to inhibition by propranolol, whereas NE-induced respiration is very sensitive, provided a first indication that NE and CL-316243 act via different receptors.

Dose-response curves of agonist-stimulated respiration. The dose-response curves for the respiratory effects of selective β1 (dutobamine), β2 (procteral), and β3 (CL-316243) agonists are compared with those of the nonselective agonists NE and isoproterenol in Fig. 2. With the exception of procteral, all the agonists tested stimulated respiration at rates similar to those elicited by NE. Other adrenergic agents, such as BRL-37344 (β3-agonist), CGP-12177 (a β1/β2-antagonist but also a β3-agonist at higher concentrations; Refs. 2, 3, 32), and epinephrine (mainly β1/β2-agonist), also maximally stimulated respiration (Table 1). NE enhanced respiration with a potency that was intermediate between that of selective β1- and β3-agonists: CL-316243 (β3; concentration eliciting 50% of maximum response (EC50) = 1.3
nM ] > BRL-37344 (β3; EC50 = 2.3 nM) > isoproterenol
(mainly β1/β2; EC50 = 5.2 nM) > NE (mainly β1/β2; EC50 = 25 nM) > epinephrine (mainly β1/β2; EC50 = 40 nM) >> dibutamine (β2; EC50 = 468 nM) >> procaterol (β2; EC50 = 12.9 µM).

The metabolic relationship between activation of lipolysis and respiration. In most cells, substrate supply does not normally control respiration, except in brown adipocytes where it is generally postulated that fatty acids released after activation of lipolysis enhance respiration by binding the mitochondrial uncoupling protein and increasing the permeability of the inner mitochondrial membrane to protons (for a review see Ref. 26). If the stimulation of respiration were a simple consequence of the activation of lipolysis by the hormone-sensitive lipase, then it would be expected that all agents activating lipolysis would also activate respiration with similar potencies (9, 38). The release of new selective β-agonists and antagonists over recent years enabled us to test this hypothesis in more detail. Using the same cell preparations and the same agonists as for respiration, we carried out a series of dose-response experiments to measure the effects of these agents on the lipolytic rates (glycerol release from the cells; Fig. 3). The responsiveness and sensitivity (EC50) of brown adipocytes for responding to various β-agonists was determined by computer analysis (SigmaPlot program) from data in Figs. 2 and 3. CGP-12177 acts as a β1-antagonist (at low concentrations) and as a selective β3-agonist (at higher concentrations) (32).

Table 1. Comparison of responsiveness and sensitivity of brown adipocytes for respiratory and lipolytic effects of adrenergic agonists

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Respiration</th>
<th>Lipolysis</th>
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<tbody>
<tr>
<td></td>
<td>Vmax, nmol O2·10^6 cells^-1·min^-1</td>
<td>EC50, nM</td>
</tr>
<tr>
<td>Basal</td>
<td>22 ± 3.2</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>CL-316243 (selective β2)</td>
<td>215 ± 13</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>BRL-37344 (selective β2)</td>
<td>205 ± 12</td>
<td>19 ± 1.3</td>
</tr>
<tr>
<td>CGP-12177 (selective β3)</td>
<td>247 ± 39.5</td>
<td>19 ± 13</td>
</tr>
<tr>
<td>Isoproterenol (nonselective)</td>
<td>229 ± 30.2</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>Noradrenaline (nonselective)</td>
<td>223 ± 23.1</td>
<td>25 ± 0.3</td>
</tr>
<tr>
<td>Epinephrine (nonselective)</td>
<td>230 ± 25.1</td>
<td>40 ± 0.4</td>
</tr>
<tr>
<td>Dibutamine (selective β3)</td>
<td>237 ± 13.5</td>
<td>468 ± 65.4</td>
</tr>
<tr>
<td>Procaterol (selective β3)</td>
<td>160 ± 6.6</td>
<td>12,900 ± 300</td>
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Values are means ± SE of 4-5 individual experiments. Responsiveness (Vmax) and sensitivity [concentration eliciting 50% of maximum response (EC50)] of brown adipocytes for responding to various β-agonists was determined by computer analysis (SigmaPlot program) from data in Figs. 2 and 3. CGP-12177 acts as a β1-antagonist (at low concentrations) and as a selective β3-agonist (at higher concentrations) (32).

Inhibition of the metabolic effects of NE by β2-adrenergic antagonists. To determine the concentrations of NE that are required to stimulate brown adipocyte thermogenesis via β1- or β3-ARs, a series of dose-response experiments was carried out, investigating the inhibitory effects of the β1-antagonist ICI-89406 (Ref. 23, and see Figs. 6B and 7B) on NE-stimulated respiration (Fig. 4) and lipolysis (Fig. 5). Increasing concentrations of ICI-89406 (from 10^-9 to 10^-5 M) shifted NE dose-response curves to the right, both for respiration and lipolysis. Notably, all dose-response curves for lipolysis were shifted to higher concentrations in comparison with the corresponding curves for respiration. This observation is consistent with the view that lipolysis drives respiration and that a relatively small stimulation of lipolysis is sufficient to maximally activate respiration (9, 10).

To better analyze the antagonist effects of ICI-89406, the respiratory and lipolytic data in Figs. 4 and 5 were plotted as percent of maximal stimulation against increasing concentrations of the β1-antagonist (Figs. 6 and 7). It can be seen that the β1-antagonist inhibited by ~50% the stimulatory effects of NE, added at a concentration of 100 nM (Fig. 6), which is the minimal concentration required to maximally stimulate respiration in the absence of antagonists (Fig. 4). However, the
effects of lower NE concentrations (25 nM), which do not maximally activate thermogenesis, were totally inhibited by ICI-89406. In contrast, the β1-antagonist barely inhibited cellular respiration in the presence of supramaximal concentrations of NE (1 µM; Figs. 4 and 6). Similar results were obtained when the effects of ICI-89406 on NE-induced lipolysis were measured (Figs. 5 and 7), providing further evidence that lipolysis and respiration represent metabolic processes that are tightly coupled.

In consideration of the observation that ICI-89406 competitively inhibited the respiratory and lipolytic effects of NE (the dose-response curves in Figs. 4 and 5 were parallel), Schild plot transformations of the data in Figs. 4 and 5 were calculated (see Figs. 4 and 5, insets). The slopes of regression lines for respiration (Fig. 4) and lipolysis (Fig. 5) were both significantly different from one, suggesting that more than one type of receptor is involved (28).

DISCUSSION

Several lines of evidence indicate that BAT lipolysis and thermogenesis are principally mediated by β1- and β3-adrenergic pathways and that β2-ARs play only a minor role in controlling these metabolic processes. Dose-response experiments revealed that β1- and β3-adrenergic agonists were very efficient for maximally stimulating brown adipocyte lipolysis and thermogenesis, whereas high concentrations of selective β2-ARs were required to partially stimulate brown adipocyte metabolism. These results are in agreement with our previous binding studies, which were performed with intact brown adipocytes rather than with membrane preparations, as it is usually done (17). In these studies, intact cells were used to avoid contamination by other membranes originating from the various cell types present in BAT. Indeed, typical brown adipocytes represent only 40% of the total cell population present in BAT. Endothelial cells forming a dense network of capillaries irrigating BAT represent another 50% of the total cells, with the remaining 10% being constituted of pericytes, interstitial precursor stem cells, brown preadipocytes, protoadipocytes, mast cells, and so forth (12,
20–22). That approach allowed us to demonstrate that the majority of β2-ARs detected in unfraccionated membranes by various groups, including ourselves, mainly originate from cells other than typical brown adipocytes, presumably endothelial cells that have been shown to contain β2- (and β1-) ARs (1, 17, 40). These studies also revealed that isolated brown adipocytes contained ~10 times more β2- than β1-ARs. However, the apparent paradox of β3-ARs is that their affinity for NE, the physiological effect of thermogenesis, is extremely low, above the micromolar range of concentrations. Plasma levels of NE vary from ~1 nM in undisturbed rats living at 25°C, to 3–4 nM at 5°C, to a maximum of 20–25 nM after exposure to extreme cold temperatures (~15°C) or decapitation (18, 30). At these concentrations, NE barely binds β2-ARs in brown adipocytes (negative log of inhibition constant K_i = 4.2), but it occupies β1-ARs that have an affinity constant in the nanomolar range (pK_i = 9.3) (17). Nevertheless, receptor occupancy merely represents the first step of a series of metabolic events leading to increased thermogenesis. The magnitude of the physiological response depends on a series of factors, such as the tightness of coupling between receptor occupancy and the adenylate cyclase system (G_i and G_o proteins), the system of protein kinases, the hormone-sensitive lipase, the amount of mitochondria per cell, the concentration of mitochondrial uncoupling protein, and so forth (15, 23–26, 29). Thus, although binding studies are useful for characterizing receptor properties, they must be completed by metabolic studies to determine the specific functions of the receptors mediating the physiological effect of NE or other agonists.

Inhibition studies using the selective β1-antagonist ICI-89406 provided good evidence that more than one β-receptor subtype was mediating the metabolic effects of NE. These studies were based on the rationale that if NE-stimulated respiration or lipolysis were exclusively controlled by β2-ARs, then one would expect that an excess of ICI-89406 would totally, or nearly totally, inhibit the metabolic effects of NE. As a matter of fact, ICI-89406 failed to totally inhibit the stimulatory effects of NE (Figs. 6 and 7), particularly at NE concentrations that maximally stimulate lipolysis or respiration (Figs. 2 and 3). Furthermore, Schild transformation (see Refs. 4 and 36) of competitive inhibition experiments analyzing the effects of increasing concentrations of ICI-89406 on NE-stimulated lipolysis or respiration (Figs. 4 and 5) revealed that the slopes of the regression lines were significantly different from one, demonstrating that more than one receptor is involved in NE action. In addition, the corresponding pA2 values for inhibition by ICI-89406 were relatively low: 8.1 for respiration and 7.2 for lipolysis. Taken as a whole, these results indicate that NE stimulates lipolysis or respiration by activating both β1- and β2-ARs, although maximal lipolysis of respiration can be reached by stimulating either β1- or β2-receptors with dobutamine or CL-316243, respectively (Figs. 2 and 3). The fact that the pA2 value for respiration is higher (8.1) than the pA2 value for lipolysis (7.2) may be interpreted as indicating that lipolysis is more controlled by the low-affinity β3-ARs than respiration. Similar observations were observed with another β1-selective antagonist, CGP-20712A (not shown). The present data also agree with a recent study showing that ICI-89406 only partially inhibits respiration stimulated by high (1 μM) NE concentrations in hamster brown adipocytes (39). However, that study did not report the effects of ICI-89406 on lower NE concentrations (nanomolar level).

The dose-response experiments comparing the effects of various agonists on lipolysis and respiration (Figs. 2 and 3 and Table 1) show that the agonists enhanced lipolysis and respiration with a similar order of potency: β3-agonists (CL-316243 and BRL-37344) > mixed agonists (isoproterenol, NE, epinephrine) > β1-agonist (dobutamine) > β2-agonist (procaterol). In general, with the exception of CGP-12177 and procat- erol, all EC50 values for lipolysis were two to four times higher than the corresponding EC50 values for respiration (Table 1), a finding suggesting that lipolysis and respiration are tightly coupled phenomena. The apparent discrepancy for procaterol probably results from the fact that this agent was unable to maximally stimulate lipolysis and respiration even when added at very high concentrations, possibly because of the scarcity of β2-ARs isolated brown adipocytes (17). On the other hand, CGP-12177 acts as a β3-antagonist at low concentrations and as a β3-agonist at higher concentrations (for references, see Ref. 32). Although it is interesting to observe that this agent was able to maximally stimulate both lipolysis and respiration, the EC50 values of CGP-12177 dose-response curves are difficult to interpret, due to the fact that this agent displays antagonist and agonist properties. Nevertheless, this latter observation demonstrates that when β2-ARs are blocked by CGP-12177, maximal respiration and lipolysis can be reached via β3-adrenergic pathways.

A tight coupling between lipolysis and respiration is supported by other observations. 1) Adenosine (1 μM), an inhibitor of lipolysis and respiration in brown adipocytes, shifts the dose-response curve for the stimulation of lipolysis and respiration by NE to higher concentrations by the same order of magnitude (~10 times) (38). 2) Propranolol inhibits NE-stimulated respiration as rapidly as it inhibits lipolysis (in <3 min; see Fig. 1 and Refs. 9, 10). 3) Long-chain fatty acids mimic the respiratory effects of NE even when endogenous adenosine 3',5'-cyclic monophosphate production and lipolysis are blocked by an excess of propranolol (9). 4) Specific inhibitors of long-chain fatty acid oxidation (methyl palmitoxirate) rapidly inhibit mitochondrial respiration (31). These observations together with the present results suggest that β1- and β3-adrenergic agonists, similarly to NE, increase mitochondrial respiration because they increase fatty acid supply to the mitochondria.

Perspective. What is the physiological function of β3-ARs? This question has been raised many times and still remains unsolved (25, 37). As pointed out in the introduction, the central problem in defining a physiological role for β3-ARs is that their affinity for NE, the
physiological mediator of thermogenesis, is very low (pKᵰ = 4.2) (17). Nevertheless, they are ~10 times more numerous (10⁵ receptors/cell) than the β₁-ARs that possess a very high affinity for NE, in the nanomolar range of concentrations. Therefore, one important question that remains to be solved concerns the physiological concentrations of NE required to elicit metabolic responses in BAT. It is known that cold exposure increases plasma NE levels from ~1 nM (basal values in conscious undisturbed rats at room temperature) up to 20–25 nM, depending on the temperature of exposure (18, 30). However, it is likely that much higher concentrations of NE occur between the sympathetic nerve varicosities and brown adipocyte plasma membranes, particularly after intensive stress. In contrast to white adipose tissue, BAT is densely innervated with sympathetic nerves that run not only along the capillaries but also between the individual adipocytes (16). Indirect evidence suggests that concentrations of NE as high as 100 nM may occur in the synaptic cleft. Depocas et al. (18, 19) have elegantly demonstrated that infused NE must reach a plasma concentration of ~100 nM to maximally activate nonshivering thermogenesis in rats maintained at room temperature. Remarkably, 100 nM is also the NE concentration that is required to maximally stimulate thermogenesis in isolated brown adipocytes (Fig. 4) (9, 10). The data of Fig. 4 clearly demonstrate that the respiratory effects of 100 nM NE can only be partially (~50%) decreased by the selective β₁-antagonist ICI-89406, whereas the effects of lower NE concentrations (which do not maximally activate thermogenesis; 25 nM) can be nearly totally inhibited by this agent. These data, combined with our binding data (17), strongly suggest that NE stimulates both β₁- and β₂-adrenergic pathways when it maximally activates brown adipocyte thermogenesis.

On the other hand, it is known that β₂-ARs may be desensitized or downregulated by chronic cold exposure (11) or prolonged exposure to β₁-agonists (13, 23). In contrast, β₂-ARs are particularly resistant to catecholamine-induced desensitization (13, 23, 34, 35). It should also be pointed out that the affinity of β₂-ARs for NE is of the same order of magnitude as the affinity of receptors for neurotransmitters such as acetylcholine (10–100 µM) (14). Thus receptors for neurotransmitters appear to have a relatively low affinity for their physiological agonists (in the micromolar range), possibly because the concentration of the agonists in the synaptic cleft may reach very high concentrations.

All these observations suggest that β₂-ARs represent the physiological receptors for NE secreted from sympathetic nerve endings when the concentration of the neurotransmitter in the synaptic cleft is high and/or when the high-affinity β₁-ARs are desensitized by prolonged sympathetic stimulation. The main role of the high-affinity β₂-ARs would be to mediate the effects of circulating NE (~25 nM; partial activation of lipolysis and thermogenesis, regulation of blood flow and cell proliferation), whereas the principal function of β₃-ARs would be to transmit the effects of NE released from sympathetic nerves when thermogenesis is maximally activated by intensive stress. In this context, it has recently been demonstrated that “cross talk” exists between β₁- and β₃-AR gene expression, the evidence being that β₁-AR mRNA (but interestingly not β₃-AR mRNA) upregulates in BAT and white adipose tissue of mice lacking β₃-ARs (37). This may explain the preponderant role of β₁-ARs in mediating the metabolic effects of NE in species lacking β₃-ARs, such as the guinea pig (6, 27).

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