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Preferential Desensitization of β- versus α_2-Adrenergic Receptors Accelerates Loss of Response to Norepinephrine in Primary Glial Cultures

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SUMMARY

The effect of norepinephrine (NE) on cAMP accumulation in primary glial cultures is mediated by two functionally opposing receptor subtypes. β-Adrenergic receptor activation increases cAMP formation, whereas simultaneous α_2-adrenergic receptor activation partially inhibits this effect. We compared desensitization of these two responses during exposure to selective agonists or NE. Pretreatment with the β-selective agonist isoproterenol (ISO) decreased responses to ISO, ISO plus the α_2-selective agonist UK 14,304 (UK), and NE. However, ISO plus UK and NE responses decreased more, relative to their control values, than did responses to ISO alone. Pretreatment with UK increased cAMP responses to both ISO and forskolin (sensitization), with little effect on α_2-mediated inhibition of these responses. Pretreatment with NE caused effects similar to those of both ISO and UK pretreatment. NE pretreatment decreased responses to ISO, ISO plus UK, and NE, sensitized responses to forskolin, and had little effect on α_2-mediated inhibition. Thus, chronic agonist exposure desensitizes β-adrenergic receptors more rapidly and at much lower concentrations than α_2-adrenergic receptors in these cultures. The continuing α_2 inhibition during diminishing β stimulation functionally accelerates the loss of the NE response.

At least nine adrenergic receptor subtypes have been identified by pharmacological and molecular cloning methods. There appear to be three major families; β_1, β_2, and β_3 receptors activate adenylate cyclase through G_s (1, 2), α_{2A}, α_{2B}, and α_{2C} receptors inhibit adenylate cyclase through G_i (3, 4), and α_{1A}, α_{1B}, and α_{1C} receptors increase intracellular Ca^{2+} (5, 6). NE activates all of these subtypes, which are often coexpressed in the same cells. Thus, the response to NE will often be due to activation of multiple receptor subtypes, which can cause opposing or redundant effects on the same signaling systems. The biological implications of the existence of multiple receptor subtypes have not yet been clarified. Chronic exposure to an agonist often results in a diminished response when cells are reexposed to the same agonist, a phenomenon referred to as desensitization. Both β (7–11) and α_2 (12–14) receptors are known to undergo various types of desensitization, although most mechanistic studies have focused on the β-adrenergic receptor system (10). We wanted to compare desensitization of each subtype expressed on the same cells. If the two subtypes exhibit differential desensitization, this could have a major impact on the loss of responsiveness to NE. Preferential desensitization of the α_2 subtype would actually increase the cAMP response by removing the inhibitory effect, whereas preferential desensitization of the β subtype would cause a rapid loss of the cAMP response.

We have been studying primary glial cultures from neonatal rat brain, where β- and α_2-adrenergic receptors exert opposing effects on cAMP accumulation. In these cultures, the cAMP response to NE is much smaller than that to the pure β agonist ISO (15–17). To determine whether these subtypes are regulated in a parallel manner, we have compared the time course and concentration dependence of desensitization of β and α_2 responses after pretreatment with selective agonists or NE.

Experimental Procedures

Materials. Timed pregnant Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN). Materials were obtained from the following sources: DMEM and calf serum, HyClone Laboratories (Logan, UT); twice crystallized trypsin, Worthington Biochemicals (Freehold, NJ); [2,8-^{3}H]adenine (10–25 Ci/mmol), New England Nuclear (Boston, MA); UK, Pfizer (Groton, CT); and forskolin, propranolol, poly-L-lysine, amphotericin B, streptomycin, penicillin G, DNase I, (−)-NE bitartrate, ISO HCl, and yohimbine HCl, Sigma Chemical Co. (St. Louis, MO).

Cell cultures. Primary glial cultures were prepared from 1-day-old

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ABBREVIATIONS: NE, norepinephrine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; DMEM, Dulbecco's modified Eagle's medium; CSd, calf serum defined; KRB, Krebs-Ringer bicarbonate buffer; ISO, (−)-isoproterenol; UK, UK 14304.

rat brains by the method of Raizada (18), as described by Wilson *et al.* (19). Brains were removed and placed in an isotonic salt solution containing 0.25 μg of amphotericin B, 100 μg of streptomycin, and 100 units of penicillin, pH 7.2. Pia mater and blood vessels were removed, and the brains were chopped into approximately 2-mm chunks. Minced tissue from 7–14 brains was suspended in 25 ml of 0.25% (w/v) trypsin in an isotonic salt solution and were placed in a 37° shaking water bath for 6 min. DNase I (160 μg) was then added to the cell suspension, which then was shaken another 6 min. Dissociated cells were collected in 10 ml of DMEM containing 10% CSd. Undissociated tissue was triturated several times, and all cells were washed with 40 ml of DMEM/CSd and centrifuged for 10 min at 1000 × *g*. Recoveries were normally 40–50 × 10^6 cells/brain. Cells were then resuspended in DMEM/CSd, and 4 ml were plated in Falcon tissue culture dishes (60 mm) that had been precoated with poly-L-lysine (2 × 10^6 cells/ml). Cells were incubated at 37°, in a humidified incubator with 5% CO_2 and 95% air, until confluent (7 days). After they reached confluence (approximately 7 days), cells were dissociated from the dishes with trypsin (0.25%), washed with DMEM/CSd, and centrifuged at 1000 × *g* for 10 min. Cells were resuspended at a density of 0.5 × 10^6 cells/ml in DMEM/CSd, and 2 ml were plated in 35-mm dishes. Cells were allowed to grow to confluence (another 4–5 days) before experimentation. Total age of the cells (after removal from brain) was typically between 12 and 21 days at the time experiments were performed.

cAMP accumulation in cultured cells. cAMP accumulation was determined by the [^{3}H]adenine prelabeling technique described by Shimizu *et al.* (20). Cells were prelabeled with [^{3}H]adenine (1 μCi/plate, in 2 ml of medium) for 2 hr. Culture medium was then removed and plates were washed with 2 ml of KRB (in mM: NaCl, 120; KCl, 5.5; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 20; glucose, 11; Ca-Na_2EGTA, 0.029) at 37°. Plates were aspirated and 1 ml of warm KRB was added. Drugs were added and the dishes were incubated for 10 min at 37°. The reaction was stopped by the addition of 0.1 ml of 77% trichloroacetic acid, and 50 μl of unlabeled cAMP were added as a carrier. Dishes were scraped with a rubber policeman, the fluid was transferred to test tubes, and each dish was washed with an additional 0.5 ml of KRB, which was added to the tubes. Samples were homogenized with a Polytron and centrifuged at 30,000 × *g* for 10 min. Then, a 50-μl aliquot of supernatant was evaluated for total tritium incorporation. [^{3}H]cAMP was isolated from the remainder of the samples by sequential DOWEX and alumina chromatography (21, 22). Results are expressed as percentage of total incorporated label converted to [^{3}H]cAMP (percentage of conversion).

Results

Time Course and Washout of cAMP Response to ISO and NE

To determine how rapidly the effects of agonists could be washed out after chronic exposure, cultures were treated with NE (1 μM) or ISO (10 nM) for 0–45 min and cAMP accumulation was measured. Some cultures were washed three times with KRB after exposure to agonist for 10 min and were terminated 0, 5, or 20 min later. Fig. 1 shows that the cAMP response to NE was much less than that to ISO at all time points; however, the response to both drugs returned to base line very quickly after washout.

Chronic ISO

Time course. Cultures were pretreated with ISO (10 nM) for 0–8 hr, washed three times with KRB, and then stimulated for 10 min with ISO (1 μM), ISO plus UK (10 μM), or NE (1 μM) (Fig. 2). Note that all responses in Fig. 2 are normalized as a percentage of the control response to each drug or combination, although responses to NE and ISO plus UK were much smaller than the response to ISO. All responses desensitized

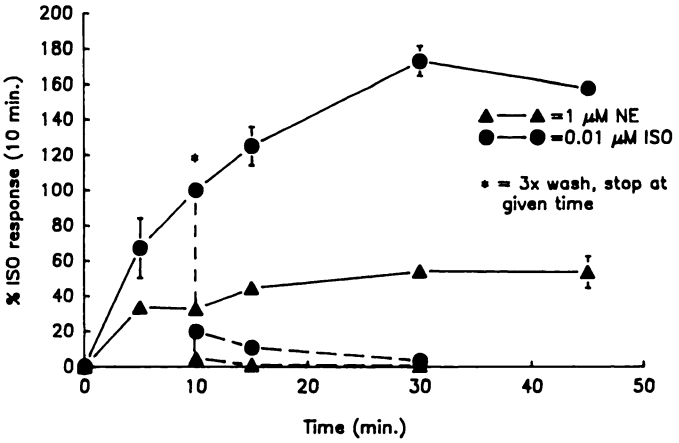

Fig. 1. Time course of onset and reversal of cAMP responses. Primary glial cultures were incubated with either 1 μM NE or 0.01 μM ISO for the indicated times and reactions were terminated or were washed after 10 min of incubation and terminated later. Data are plotted as a percentage of the cAMP response to ISO after 10 min of incubation. Each *point* represents the mean ± standard error of four culture dishes from independent experiments.

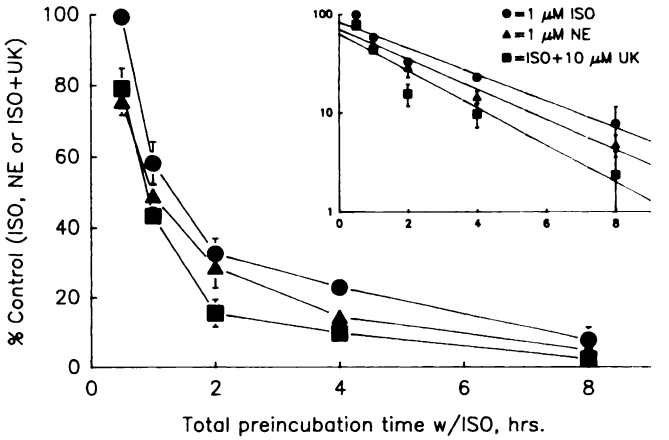

Fig. 2. Time course of desensitization of cAMP responses to agonists after ISO pretreatment. Cultures were preincubated with 0.01 μM ISO (0.1 μM ascorbate present) for the indicated times, washed three times with KRB, and stimulated with either 1 μM ISO, ISO plus 10 μM UK, or 1 μM NE, for 10 min. Responses are plotted as a percentage of the control cAMP response for each drug or combination of drugs. Control values were as follows: basal, 0.03 ± 0.008%; ISO, 2.9 ± 0.52%; NE, 0.44 ± 0.08%; and ISO plus UK, 0.77 ± 0.14%. Each *point* represents the mean ± standard error of four independent determinations. *Inset:* semilog plot of the same data.

rapidly, with a half-maximal loss occurring after 1-hr pretreatment. After 8-hr pretreatment, responses to all three drugs or combinations were virtually abolished. Responses to NE and ISO plus UK fell more rapidly, as a percentage of the control response ($p < 0.05$), than did the response to ISO alone (Fig. 2).

Concentration dependence. The concentration dependence of desensitization caused by ISO is shown in Fig. 3. Preincubation with increasing concentrations of ISO (with 0.1 μM ascorbic acid) for 14 hr decreased responses to ISO, ISO plus UK, and NE. Pretreatment with as little as 0.1 nM ISO caused significant decreases in each response, and pretreatment with 5 nM ISO abolished all responses (Fig. 3, *upper*). Normalization of the data to a percentage of the control response (Fig. 3, *lower*) shows that the concentration dependence was not the

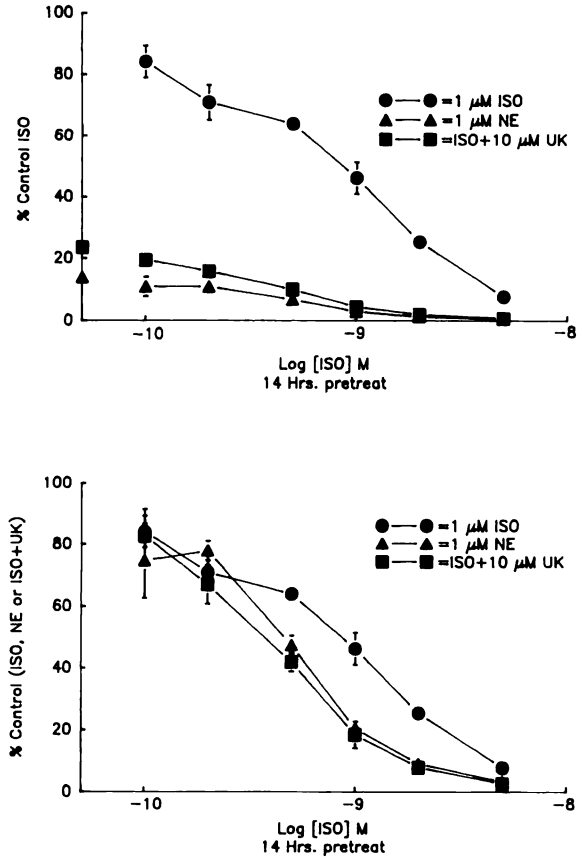

Fig. 3. Effect of ISO pretreatment on cAMP responses to ISO, ISO plus UK, and NE. Cultures were pretreated with the indicated concentrations of ISO (0.1 μM ascorbate present) for 14 hr, washed, and stimulated with either 1 μM ISO, ISO plus 10 μM UK, or 1 μM NE, for 10 min. Data are expressed as a percentage of the control cAMP response to ISO (*upper*) or as a percentage of control cAMP responses to each drug or combination of drugs (*lower*). Control values were as follows: basal, 0.03 ± 0.002%; ISO, 3.7 ± 0.24%; NE, 0.53 ± 0.08%; and ISO plus UK, 0.89 ± 0.04%. Each *point* represents the mean ± standard error of four independent determinations.

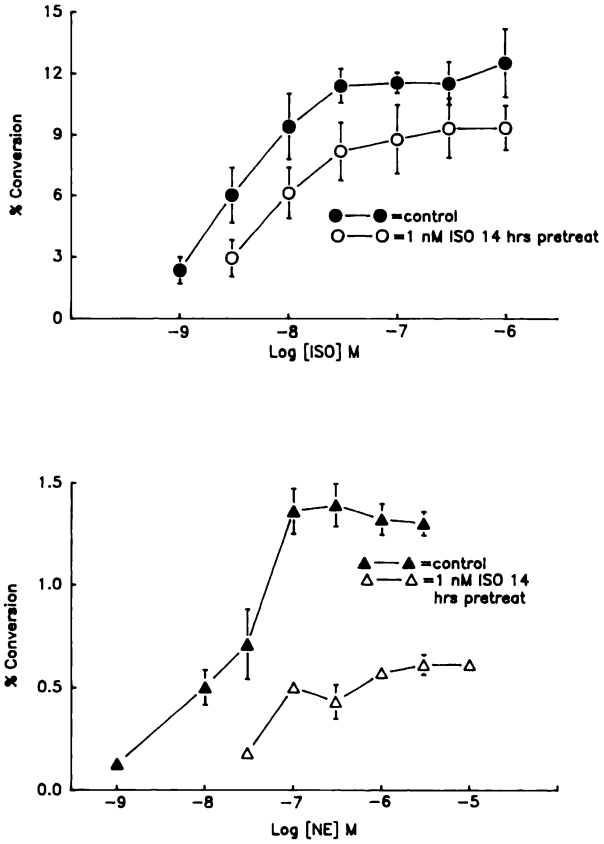

Fig. 4. Effect of ISO pretreatment on concentration-response curves for ISO (*upper*) or NE (*lower*). Cultures were pretreated with 1 nM ISO (0.1 μM ascorbate present) for 14 hr, washed, and stimulated with the indicated concentrations of ISO or NE, for 10 min. Each value is the mean ± standard error of four independent determinations.

same for each drug or combination. Responses to NE or ISO plus UK were lost at lower pretreatment concentrations than were responses to ISO. Calculation of the $-\log EC_{50}$ for ISO in desensitizing the different responses gave values of 9.15 ± 0.072 for ISO, 9.36 ± 0.033 for NE, and 9.47 ± 0.072 for ISO plus UK. The $-\log EC_{50}$ values for both NE and ISO plus UK were significantly different from that for ISO ($p < 0.05$). Thus, approximately 2–3-fold lower pretreatment concentrations of ISO were needed to desensitize the response when α_2 receptors were also being activated.

Shift in agonist concentration dependence. Concentration-response curves for ISO- and NE-stimulated cAMP accumulation were compared in control cultures or cultures pretreated with 1 nM ISO for 14 hr (Fig. 4). The maximal response to ISO (Fig. 4, *upper*) decreased 23 ± 9%, whereas the EC_{50} shifted slightly to the right (4.4 to 8.4 nM), consistent with the presence of a small β-adrenergic receptor reserve. The maximal response to NE (Fig. 4, *lower*) showed a much larger decrease, 62 ± 5%, whereas the EC_{50} shifted slightly more (40 to 220 nM).

Effect on forskolin and α_2 inhibition. Pretreatment with ISO (14 hr, 0.1 μM ascorbate) caused a small inhibition of the subsequent response to forskolin (1 μM), with a maximal decrease of 31 ± 2.5% at 0.1 μM ISO (Fig. 5). At the highest ISO

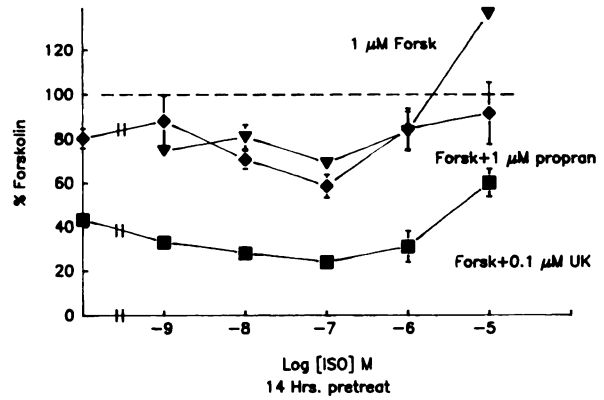

Fig. 5. Effect of ISO pretreatment on cAMP responses to forskolin and forskolin plus UK. Cultures were pretreated with the indicated concentrations of ISO (0.1 μM ascorbate present) for 14 hr, washed, and stimulated with either 1 μM forskolin (*Forsk*), forskolin plus 0.1 μM UK, or forskolin plus 1 μM propranolol (*propran*), for 10 min. Control values were as follows: basal, 0.06 ± 0.005%; forskolin, 2.3 ± 0.32%; and forskolin plus UK, 1.0 ± 0.13%. Each value is the mean ± standard error of four independent determinations.

pretreatment concentration (10 μM), the forskolin response was increased (36 ± 1.3%). However, this increase was blocked by propranolol (1 μM), suggesting that residual ISO was probably potentiating the forskolin response. ISO concentrations less than 1 nM potentiate the cAMP response to 1 μM forskolin as much as 2-fold (data not shown), suggesting that even a

100,000-fold dilution would not be sufficient to wash out completely the highest ISO pretreatment concentration examined.

Inhibition of the forskolin response by UK was slightly increased by ISO pretreatment (Fig. 5). The effect of forskolin plus UK was maximally decreased by 38% after 0.1 μM ISO pretreatment. This response also increased at the highest pretreatment concentration, probably due to residual ISO.

Chronic UK

Effect on responses to forskolin and ISO. Pretreatment with increasing concentrations of the α_2-selective agonist UK increased the cAMP response to 1 μM forskolin (Fig. 6, *upper*), except at higher concentrations. The response began to decrease at pretreatment concentrations of 10 and 100 μM. This decrease is probably due to residual UK in the medium, because it was eliminated by yohimbine (10 μM). The cAMP response to forskolin plus yohimbine increased to a maximum of 3-fold over control. Pretreatment with UK also increased the cAMP response to ISO (1 μM) at all but the highest pretreatment concentration (Fig. 6, *lower*). Again, the decrease observed at the highest UK concentration is probably due to residual UK, because it was reversed by yohimbine. The cAMP response to ISO plus yohimbine increased a maximum of 1.9-fold, compared with control.

Effect on α_2-mediated decreases in cAMP. The ability of α_2 receptors to inhibit β-adrenergic receptor- or forskolin-stimulated cAMP responses was examined after chronic exposure to the α_2-selective agonist UK. In general, there was little effect on α_2-mediated inhibition. Although cAMP responses to NE (Fig. 7, *upper*), ISO plus UK, and forskolin plus UK (data not shown) increased substantially after pretreatment with increasing concentrations of UK, this appeared to be due primarily to the sensitization of β-adrenergic receptor- and forskolin-stimulated cAMP responses described above. There was little desensitization of the α_2 inhibition, as seen in Fig. 7 (*lower*), where data are expressed as a percentage of the control response to ISO (plus yohimbine, to block residual UK) or forskolin (plus yohimbine) alone. However, inhibition of the ISO response by UK decreased slightly, from 34 ± 1% in control cultures to 56 ± 3.7% after 1 μM UK pretreatment.

Shift in agonist concentration dependence. Although there was little or no loss in maximal α_2-mediated inhibition of cAMP responses after chronic UK pretreatment, we examined the possibility of a reduction in receptor reserve. Pretreatment with 1 μM UK produced an increased response to ISO, as expected (Fig. 8, *inset*), but also caused a significant 5-fold shift to the right in the EC_{50} for subsequent inhibition by UK (Fig.

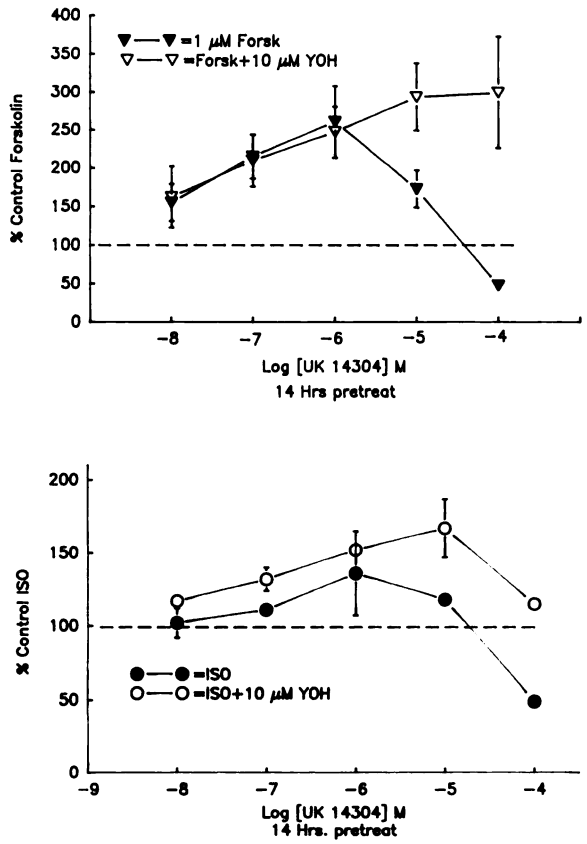

Fig. 6. Effect of UK pretreatment on cAMP responses to ISO and forskolin. Cultures were pretreated with the indicated concentrations of UK for 14 hr, washed, and stimulated with 1 μM forskolin (*Forsk*) with or without 10 μM yohimbine (*YOH*) (*upper*) or 1 μM ISO with or without yohimbine (*lower*), for 10 min. Data are calculated as a percentage of the control ISO or forskolin responses. Control values were as follows: basal, 0.03 ± 0.002%; forskolin, 1.2 ± 0.30%; and ISO, 3.9 ± 0.71%. Each *point* is the mean ± standard error of four independent determinations.

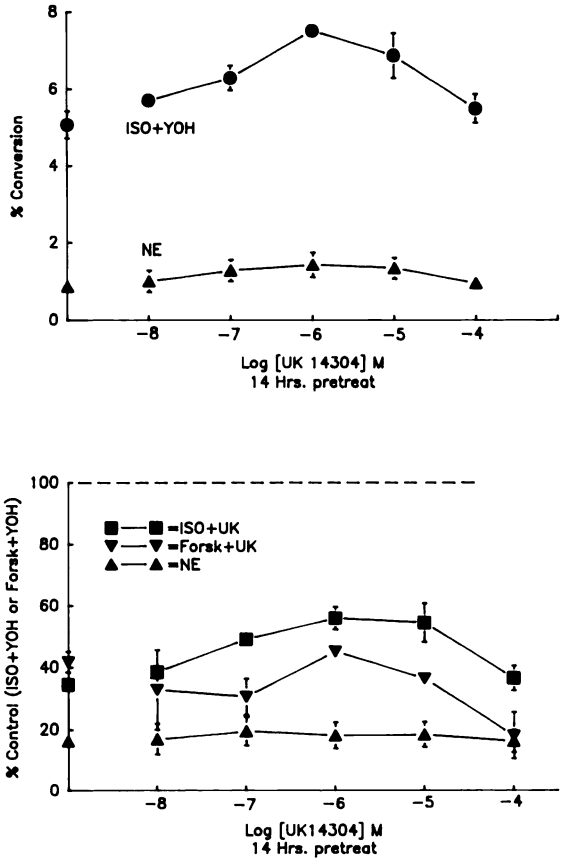

Fig. 7. Effect of UK pretreatment on α_2-mediated inhibition of cAMP responses. Cultures were pretreated with the indicated concentrations of UK, washed, and stimulated with 1 μM ISO with or without 10 μM UK, 1 μM forskolin (*Forsk*) with or without 0.1 μM UK, or 1 μM NE in the presence or absence of 10 μM yohimbine (*YOH*), for 10 min. *Upper*, actual data for responses to NE and ISO plus yohimbine. *Lower*, data normalized as a percentage of the control ISO plus yohimbine (ISO plus UK and NE) or forskolin plus yohimbine (forskolin plus UK) cAMP response. Each value is the mean ± standard error of four independent determinations.

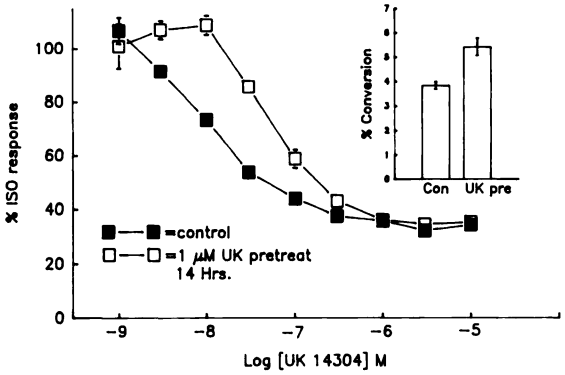

Fig. 8. Effect of UK pretreatment on concentration-response curve for inhibition of the ISO response by UK. Cultures were pretreated with 1 μM UK for 14 hr, washed, and stimulated with 1 μM ISO plus the indicated concentrations of UK, for 10 min. *Inset*, actual responses to ISO in control and UK-pretreated cultures. The other data are normalized as a percentage of the control ISO response for each condition. Each value is the mean ± standard error of four independent determinations.

8). Subsequent studies using alkylating agents have shown that the α_2 receptor reserve in this system is between 5- and 10-fold.[1] Thus, some desensitization of the α_2 response is occurring, but only at high agonist concentrations and long exposure times.

Chronic NE

Effect on responses to forskolin and forskolin plus UK. Pretreatment with NE yielded changes in cAMP responses similar to those observed with both ISO and UK pretreatment. Fig. 9 (*upper*) shows that NE pretreatment increased the response to forskolin (1 μM) by up to 3-fold. The β-selective antagonist propranolol had a small effect on this potentiation only at the highest NE pretreatment concentration, suggesting that some residual NE remained to potentiate the forskolin response. The ability of UK to inhibit the forskolin response was reduced slightly, from 69% inhibition in control cultures to 49% inhibition after pretreatment with 100 μM NE (Fig. 9, *lower*). The response to forskolin did not appear to be attenuated by residual α_2 receptor activation by NE, because yohimbine had no effect on response (data not shown).

Effect on responses to ISO, ISO plus UK, and NE. Pretreatment with NE caused concentration-dependent decreases in cAMP responses to ISO, ISO plus UK, and NE, reminiscent of those occurring after ISO pretreatment (Fig. 10). Pretreatment with 1 μM NE virtually eliminated responses to all agonists. As observed above with ISO-pretreated cultures, responses to NE or ISO plus UK were lost at lower pretreatment concentrations than were responses to ISO. Calculation of the $-\log EC_{50}$ for NE in desensitizing the different responses gave values of 7.22 ± 0.157 for ISO, 8.13 ± 0.048 for NE, and 8.04 ± 0.110 for ISO plus UK. The $-\log EC_{50}$ for both NE and ISO plus UK were significantly different from that for ISO ($p < 0.01$). Thus, approximately 7–9-fold lower pretreatment concentrations of NE were needed to desensitize the response when α_2 receptors were also being activated.

Discussion

Chronic agonist exposure often results in desensitization to further agonist activation. This phenomenon has been best studied using the β-adrenergic receptor coupled to adenylate cyclase as a model system (10). Although a number of complex mechanisms are involved, chronic agonist treatment generally causes a rapid uncoupling of the receptor-guanine nucleotide-binding protein-adenylate cyclase complex and sequestration of receptors from the cell surface (7–11). Receptor density is subsequently lost at a substantially slower rate.

Less is known about desensitization of α_2-adrenergic receptors. However, chronic agonist exposure also results in desensitization of α_2 receptors in some cell lines (13, 14, 23). Rapid desensitization of the platelet aggregatory response to α_2 receptor activation has also been observed, but without subsequent loss of receptor density (24, 25) or impairment of α_2 receptor/adenylate cyclase coupling (12).

We have examined the effects of chronic adrenergic agonist exposure on cAMP responses in primary glial cultures. NE-stimulated cAMP accumulation in this and other primary glial culture systems is mediated through activation of both β- and α_2-adrenergic receptors (15–17, 26). Although desensitization of both β- and α_2-adrenergic receptors has been characterized previously, few studies have compared the regulation of these

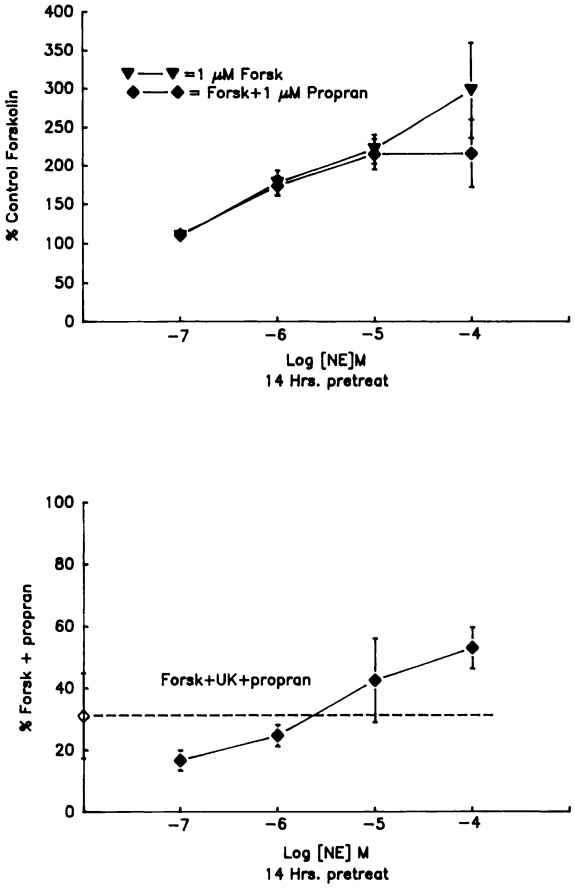

Fig. 9. Effect of NE pretreatment on cAMP responses to forskolin (*Forsk*) and forskolin plus UK. Cultures were pretreated with the indicated concentrations of NE (0.1 μM ascorbate present), washed, and stimulated with 1 μM forskolin or forskolin plus 0.1 μM UK, with or without 1 μM propranolol (*propran*), for 10 min. Data are expressed as a percentage of the control forskolin response (*upper*) or a percentage of the forskolin plus propranolol response (*lower*). Control values were as follows: basal, 0.04 ± 0.005%; forskolin, 2.1 ± 0.16%; and forskolin plus propranolol, 2.4 ± 0.21. Each value is the mean ± standard error of four independent determinations.

[1] B. N. Atkinson and K. P. Minneman, unpublished observations.

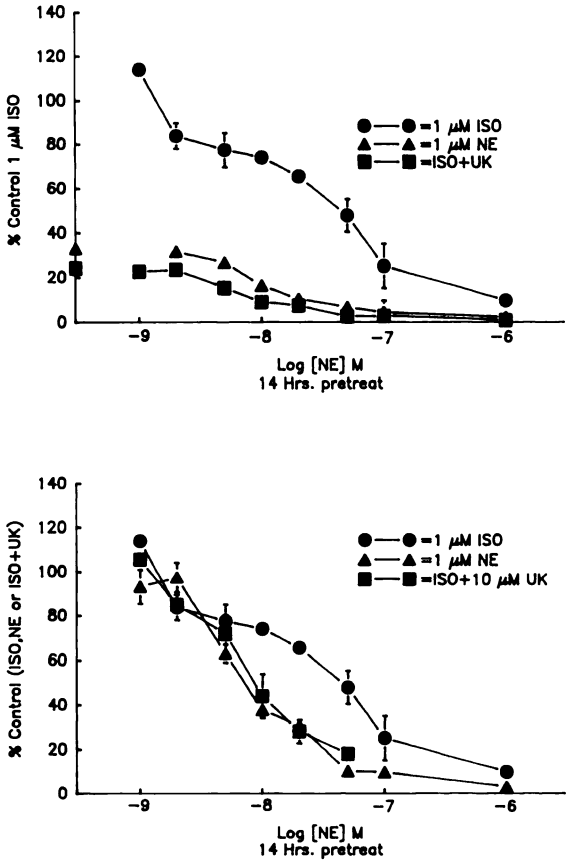

Fig. 10. Effect of NE pretreatment on responses to ISO, ISO plus UK, and NE. Cultures were pretreated with the indicated concentrations of NE (0.1 μM ascorbate present), washed, and stimulated with 1 μM ISO, ISO plus 10 μM UK, or 1 μM NE, for 10 min. Data are expressed as a percentage of the 1 μM ISO response (*upper*) or as a percentage of the control response for each drug or combination of drugs (*lower*). Control values were as follows: basal, 0.02 ± 0.001%; NE, 2.4 ± 0.19%; ISO, 9.8 ± 0.53%; and ISO plus UK, 3.6 ± 0.14%. Each value is the mean ± standard error of four independent determinations.

receptors and the response to NE after chronic exposure in the same system.

The cAMP response after chronic exposure to NE depends greatly on the relative rates and concentration dependence of desensitization of each subtype. For example, if α_2 receptors desensitized before β receptors, the NE response might first rise as the α_2 inhibition was lost and subsequently decline as the β stimulation diminished. Conversely, the NE response might simply decline as both the β and α_2 receptors were lost. Alternatively, there might be an interactive effect of the two subtypes. In fact, Northam and Mobley (27) reported that the α_2 agonist clonidine partially reversed ISO-induced desensitization of β-adrenergic receptors in primary astrocyte cultures. In this case, concomitant stimulation of both β and α_2 receptors with NE might not cause any desensitization.

As expected, the response to ISO decreased in a time- and concentration-dependent fashion after chronic ISO pretreatment. Responses to NE and ISO plus UK also decreased, but more quickly and at lower ISO concentrations than did the response to ISO. This was also apparent in the concentration-response curves for ISO- and NE-stimulated cAMP responses after ISO pretreatment. Although each curve was shifted slightly to the right, consistent with a small β receptor reserve, the maximal ISO response decreased only 23% with 1 nM ISO pretreatment, whereas the maximal NE response decreased by 62%. These results are consistent with a rapid and complete desensitization of β-adrenergic receptors during chronic agonist treatment of these cultures.

Surprisingly, pretreatment with the α_2 agonist UK had little or no effect on maximal α_2-mediated inhibition of the response to either forskolin or ISO. However, the concentration-response curve for UK inhibition of the ISO response was shifted almost 5-fold to the right after pretreatment with high concentrations of UK. This suggests the presence of a small receptor reserve, which is reduced by chronic UK pretreatment. Thus, although there was little or no decrease in the maximal α_2 inhibition, UK pretreatment did cause some desensitization of the α_2 response. In addition, pretreatment with UK increased both β-adrenergic receptor- and forskolin-stimulated cAMP accumulation, in agreement with previous studies (28–30). The mechanism for this apparent "sensitization" is not understood. Thus, the α_2 receptors in these cultures are quite resistant to desensitization, and only small effects are seen after chronic treatment with high agonist concentrations.

Pretreatment with NE produced effects similar to those caused by pretreatment with both β- and α_2-selective agonists. The response to forskolin was increased, as was observed with UK pretreatment. The response to ISO was decreased, and the responses to NE and ISO plus UK decreased more, relative to their control responses, than did that to ISO alone. Again, there was little evidence of α_2 receptor desensitization with NE pretreatment, because UK inhibition of forskolin-stimulated cAMP response was only slightly attenuated, even at the highest pretreatment concentrations. Interestingly, the difference between loss of the β response alone and that of the combined β plus α_2 responses was substantially greater during chronic NE pretreatment than with chronic ISO pretreatment.

Although it is feasible to measure β-adrenergic receptors in these preparations (31), due to the lack of a suitable high specific activity ligand it is necessary to passage the cells to obtain sufficient material for measuring α_2 receptor density (32). Because we are interested in the functional interactions between the two receptor subtypes, and because it has been shown that binding sites are usually lost at a substantially slower rate than are responses, we did not measure receptor density in these cultures. Presumably, both β- and α_2-adrenergic receptor binding sites would be lost relatively slowly and at high agonist concentrations. For example, Baker *et al.* (31) reported that exposure of similar cultures to 10 μM isoproterenol for 24 hr resulted in only an 82% loss of β-adrenergic receptor binding sites, whereas we found that exposure to 0.003 μM isoproterenol for 14 hr completely abolished the response to isoproterenol.

Thus α_2- and β-adrenergic receptors respond very differently to chronic agonist exposure in this cell preparation. Using either selective agonists or NE, β-mediated responses were rapidly lost at very low agonist concentrations, whereas α_2-mediated responses were only slightly diminished, even at agonist pretreatment concentrations several orders of magnitude higher than those necessary to completely desensitize the β response. This differential desensitization seemed to have an important functional consequence, in that the response to NE (involving both β and α_2 components) was lost more rapidly and at lower pretreatment concentrations than was the response to ISO. It seems likely that this is due to a continuing

α_2 inhibition occurring during loss of the β stimulation, resulting in a more dominant ratio of α_2 to β stimulation by NE in the partially desensitized cultures.

These results demonstrate that the adrenergic receptors mediating the cAMP response to NE in primary glial cultures are regulated differently in response to chronic agonist exposure, with unexpected functional consequences for subsequent response to NE. Further investigation of how differential regulation of subtypes within each of these adrenergic receptor families (β_1, β_2, β_3; α_{2A}, α_{2B}, α_{2C}) affects the response to NE will be needed to understand the implications of responses mediated through simultaneous activation of multiple subtypes by a single neurotransmitter.

Acknowledgments

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