Tumor Necrosis Factor \( \alpha \) Enhances Nicotinic Receptor Up-regulation via a p38\(^{MAPK} \)-dependent Pathway*

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A response by key neuronal nicotinic acetylcholine receptors (nAChRs) to sustained nicotine exposure is up-regulation. Although this unusual receptor characteristic contributes to processes ranging from aging to addiction, the normal physiological reason for this response is unknown. We find that up-regulation of \(^{1}H\)epibatidine binding and function in HEK293 cells stably expressing \(\alpha4\beta2\)-nAChR is significantly enhanced by co-application of the proinflammatory cytokine, tumor necrosis factor \(\alpha\). The mechanism of tumor necrosis factor \(\alpha\)-enhanced up-regulation requires transcription, new protein synthesis, and signaling through p38\(^{MAPK} \) as demonstrated by complete inhibition using SB 202190. This finding extends the possibilities for nAChR-inflammatory interactions in normal physiological processes and offers novel insights into endogenous mechanisms that can modify up-regulation.

Neuronal nicotinic acetylcholine receptors (nAChRs) play an important role in modulating normal neurotransmission in the central nervous system. These receptors also have a direct impact upon behavioral and physiological pathologies ranging from addiction to their early and selective loss in Alzheimer disease (1). Addiction to nicotine correlates with the curious trait of up-regulation in response to chronic exposure to receptor ligands (2–4). The majority of high affinity nicotine receptors that undergo up-regulation in the mammalian brain are composed of at least nAChR \(\alpha4\) and \(\beta2\) subunits as demonstrated by high affinity ligand binding and genetic studies (1, 5–7). Although multiple mechanisms contribute to up-regulation (1, 8, 9), the normal physiological reason for this response is poorly understood despite its being conserved in animals ranging from Caenorhabditis to mammals. Since most of these organisms have never been exposed to nicotine either acutely or at any time throughout their evolutionary history, up-regulation probably reflects a normal physiological response preserved by processes of natural selection. It is also an intrinsic property of nAChRs composed of \(\alpha4 + \beta2\) subunits (termed \(\alpha4\beta2\)-nAChR; see Refs. 10 and 11 for additional nomenclature). Even when expressed in heterologous systems, such as human embryonic kidney cells, this receptor responds to nicotine with pharmacokinetics that are almost identical to those of the mammalian forebrain (1, 12).

Possible reasons for endogenous up-regulation of nAChR may involve the recent recognition of the participation of nicotinic receptors in regulating proinflammatory processes. In this context, \(\alpha7\)-nAChR has received the greatest attention since its antagonism of the proinflammatory cytokine, TNF\(\alpha\), was documented over a decade ago (13). Subsequent investigations have supported the physiological relevance of this interaction in regulating numerous proinflammatory processes (14). However, a more complex interaction between nAChRs and inflammation is suggested by studies using both tissue culture (13, 15, 16) and animal models (14, 17). For example, proinflammatory cytokines can impact upon nAChR expression through promoting efficient receptor assembly and altering relative subunit composition of mature nAChRs when HEK293 (293) cells are co-transfected with cDNA encoding \(\alpha4\) and \(\beta2\) and/or \(\beta4\), respectively (16). In these earlier studies, we did note a small but persistent increase of \(^{1}H\)epibatidine (\(^{1}H\)Eb) binding in 293 cells transiently expressing \(\alpha4\beta2\)-nAChR that were also treated with TNF\(\alpha\) (16). This has been examined further with 293 cells stably expressing nAChRs. Our findings show that the proinflammatory cytokine, TNF\(\alpha\), dramatically enhances \(\alpha4\beta2\)-nAChR up-regulation induced by nAChR ligands, including nicotine, cytisine, and carbachol. The pathway to enhancement of up-regulation is both actinomycin D- and cycloheximide-sensitive, and it is inhibited by SB 202190, a highly specific inhibitor of p38\(^{MAPK} \). These findings offer a novel insight into how proinflammatory cytokines can impact upon mechanisms of up-regulation and strongly suggest that reciprocal regulatory interactions between the nAChR and inflammatory systems are likely.

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§ The abbreviations used are: nAChR, neuronal nicotinic acetylcholine receptor; 293, HEK293; Eb, epibatidine; TNF\(\alpha\), tumor necrosis factor \(\alpha\); TNF\(\beta\), tumor necrosis factor receptor; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.

*The 293 cell lines stably co-transfected with nAChR subunits \(\alpha4 + \beta2\), \(\alpha4 + \beta4\), \(\alpha3 + \beta2\), or \(\alpha3 + \beta4\), respectively, were generously provided by Drs. Ken Kellar and Yingxian Xiao (Department of Pharmacology, Georgetown University). These cells were maintained as described (12, 18, 19). Additional analysis of these cells by real time PCR showed expression of RNA encoding the TNFR sub-
type 1 (cycle thresholds of 22) and the less abundant TNFR type 2 (cycle threshold of 35) compared with β-actin (cycle threshold of 17; not shown). Application of TNFα (human recombinant TNFα; BioSource) to these cells (25 ng/ml for 2–4 h) revealed that RNA encoding the inducible form of cyclooxygenase, COX2, was elevated 3-fold, demonstrating that TNFα stimulates cellular responses in these cells (not shown). The same assays revealed no evidence for the expression of the acetylcholine-synthesizing enzyme, choline acetyltransferase, or other human nAChR subunits (not shown).

Radioligand Binding—The binding of [3H]Eb to cell membrane preparations was done essentially as described (12, 16), with the following modifications. Cells were distributed into 100-mm culture dishes and treated (e.g. with nicotine) 48 h later. Cells were harvested 18–24 h after treatment, at which time the cultures were 50–75% confluent, into 50 mM Tris buffer (pH 7.4, 4 °C), pelleted, resuspended, and homogenized. Cellular debris and nuclei were removed by low speed centrifugation (100 × g, 5 min), and the supernatant was collected and centrifuged (20,000 × g for 10 min) to pellet remaining membranes for ligand-binding assays. For binding assays, 5 μg of membrane was incubated with 5 nM [3H]Eb for 2–4 h at room temperature (~25 °C). Nonspecific binding was assessed by adding 500 μM nicotine hydrogen tartrate (Sigma) for 30 min before the addition of [3H]Eb to block specific binding. Samples were tested in triplicate. Ligand was separated from free ligand by vacuum filtration through Whatman GF/C filters and prepared for scintillation counting. Specific binding was defined by averaging the total binding minus the nonspecific (nicotine-blocked) binding. Data were analyzed using Prism 3 (GraphPad Software Inc., San Diego, CA) as described (12, 18, 19).

Calcium Imaging—Fura-2/AM calcium imaging was done essentially as before (20). Cells were grown on glass coverslips coated with poly-l-lysine and laminin and washed four times with Hanks’ solution (with 1.3 mM CaCl2 (Invitrogen)) over 20 min, and fresh buffer was added containing 500 μM nicotine (see “Experimental Procedures”). Nicotine was rapidly applied directly onto the cells using a fixed pipette or a picospritzer. The cell response (change in the 340/380 nm ratio) was then recorded. Nicotine was then added a second time, and the measurement was repeated before adding 1 mM acetylcholine to activate muscarinic receptors as a positive control for cell responsiveness (~98% in each test group). Recordings were prepared from multiple coverslips in independent platings, which were scored for responsiveness. A responsive cell was defined as having a change in the peak intensity of the 340/380 nm ratio of 0.01 units from resting upon nicotine application and that failed or exhibited a very poor response to the second application (desensitization). Primary data were analyzed using Excel.
TNFα Enhances Nicotinic Receptor Up-regulation

A dose-response assay for α4β2-nAChR up-regulation of [3H]Eb binding sites was shown. The optimal concentration inducing the small but significant up-regulation of [3H]Eb sites was 12.5–25 ng/ml, with the 25 ng/ml amount providing the greatest consistency among all experiments. C, the dose response of TNFα when nicotine is a constant at 1 μM. Enhancement of up-regulation [3H]Eb sites was optimal at 1 μM nicotine plus 25 ng/ml TNFα. D, cell density influences the magnitude of TNFα enhancement of up-regulation. Cells were plated to reach the confluence indicated at harvest. Basically, ligand-mediated up-regulation is not statistically different from the control at increasing confluence. However, the magnitude of TNFα enhancement of up-regulation decreases from 1.8-fold (50% confluence) to an average of ~1.4-fold as densities exceed 50% confluence. Student’s t test was used; *, p < 0.05; **, p < 0.01. Nic, nicotine; Con, control.

An important variable in tissue culture experiments is cell culture density. This is particularly true when variables such as cell cycle, nutrients, pH, and cell-cell contact (that may also affect accessibility by ligands to receptors) influence the experimental results. The influence of cell density on TNFα-enhancement of nicotine-mediated up-regulation was examined in cultures that were plated to reach confluence of 100, 75, 50, and 25%, respectively, following a total of 36 h in culture and 24 h after treatment with nicotine, TNFα, or both agents as above. The results show (Fig. 2D) that cell cultures at ~50% confluence display the greatest enhancement by TNFα of up-regulation (1.8-fold). There was, however, a decline in the amount of TNFα enhancement of up-regulation with increasing cell density (~1.4-fold). This result indicates that cell culture conditions influence the ability of TNFα to enhance nicotine-mediated up-regulation. This finding is consistent with cell density influencing nAChR expression by transfected cultured cells. For all experiments reported, culture density was ~50% confluence at the time of harvest.

TNFα Enhancement of Nicotine-mediated Up-regulation Requires New RNA Transcription and Protein Synthesis—The mechanism of nicotine plus TNFα-enhanced up-regulation was examined next. To begin, saturation binding of [3H]Eb over a concentration range of 1–5000 pm for each treatment group was measured (Fig. 3A). For control cells, the [3H]Eb-specific binding was 537 ± 140 fmol/mg, and the saturable best fit was to a single high affinity site (control mean Kd of 53 ± 14 pm) as calculated using Prism 3.0 software (12). Nicotine competition curves averaged from three independent experiments (Fig. 3B; nicotine binding competition against 500 pm [3H]Eb) show that nicotine alone had no significant influence on the results (not shown). Therefore, these data support a model where combining nicotine and TNFα up-regulation by co-exposure is related to changes in receptor number.

How TNFα enhances nicotine-mediated up-regulation of α4β2-nAChR [3H]Eb binding sites was examined further. Although previous reports show ligand-mediated up-regulation of nAChRs to be independent of transcription (1), how TNFα enhancement of up-regulation occurs is unknown. The first measurement determined if intact cells were required for TNFα to enhance nicotine-mediated up-regulation. For this experiment, a crude membrane fraction of untreated cells was prepared as described for binding assays. Equal aliquots of these preparations were treated with saline, 1 μM nicotine, 25 ng TNFα, or both nicotine and TNFα and incubated for 37 °C for 24 h with gentle rocking. Membranes were then collected by centrifugation and washed, and radioligand-binding measurements were performed. The results (Fig. 3C) show that incubation of membranes with TNFα alone had no effect on [3H]Eb binding, although a small but significant increase was measured in nicotine-treated samples and those with nicotine plus TNFα. This persistent increase is consistent with reports of up-regulation through mechanisms that influence receptor affinity for ligand (9). Nevertheless, the changes in ligand binding were well below the 4–6-fold change expected from whole cell preparations, and no TNFα enhancement of this up-regulation effect was present, indicating that TNFα acts on up-regulation through mechanisms requiring intact cells.

The time course of TNFα-enhanced up-regulation was examined to determine when TNFα-enhanced up-regulation begins. These experiments were done as above, only cells were harvested at various times after the addition of nicotine and/or TNFα. As shown in Fig. 3D, a small enhancement by TNFα of nicotine up-regulation was observed within 2 h of treatment; however, significant enhancement of up-regulation was not observed until ~8 h post-treatment. This result indicates that
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FIGURE 3. TNFα enhancement of up-regulation is dependent upon increased receptor number and is sensitive to inhibitors of transcription and translation. A, a saturation binding of [3H]Eb to membrane homogenates (5 μg) prepared from α4β2-αChRs-expressing cells treated for 24 h with saline (control), 1 μM nicotine (Nic), TNFα (25 ng/ml), or both nicotine and TNFα. Saturation binding assays were carried out as described under “Experimental Procedures” for a [3H]Eb concentration range of 1–5000 pM. Binding data were analyzed by nonlinear least square regressions using Prism 3 software and the one-site saturation binding model. The data shown are from a single representative experiment of three that gave essentially equivalent results. B, ligand binding competition profiles for nicotine on membrane homogenates from cells expressing α4β2-nAChRs and [3H]Eb. The data were analyzed by the nonlinear least square regression method using Prism 3 software as reported elsewhere (12, 19), and they reflect results from one experiment that is typical. Error bars were omitted for clarity. A single curve fit (for nicotine-treated cells) is shown, but curve fits among different treatment groups were essentially identical when all experiments (n = 3) were averaged. C, the results of [3H]Eb binding to membrane homogenates prepared from control α4β2-nAChRs cells, which were then treated for 24 h with saline (control), 1 μM nicotine, TNFα (25 ng/ml), or both nicotine and TNFα. The results are shown as the ratio of specific ligand binding to the control, which is 1.0. D, cells expressing α4β2-nAChRs were treated with 1 μM nicotine (open bars) or 1 μM nicotine plus 25 ng/ml TNFα (gray bars) for the times indicated. The -fold change in [3H]Eb binding site density over controls is shown. Highly significant (p < 0.01) TNFα enhancement of up-regulation is apparent by 8 h postincubation. E, the effect of 10 μM actinomycin D on TNFα enhancement of [3H]Eb site up-regulation by nicotine. All values are normalized to the relevant control set (saline or saline plus actinomycin D-only treatments). F, the effect of 10 μM cycloheximide on inhibiting the up-regulation of [3H]Eb sites. All values are normalized to the control (saline, saline plus cycloheximide-only treatments). Both actinomycin D and cycloheximide inhibit TNFα-enhanced up-regulation without decreasing nicotine-mediated up-regulation relative to control samples. Error bars, ± S.E.; Student’s t test; *, p < 0.05; **, p < 0.01. N.S., not significant.

the impact of TNFα on up-regulation is not likely to be through modifications of preexisting protein pools. To test this, we next examined the influence of the transcriptional inhibitor, actinomycin D, on TNFα-enhanced up-regulation. In these experiments, [3H]Eb binding of membranes isolated from cells treated with nicotine, TNFα, nicotine plus TNFα, or saline for 18 h, either in the presence or absence of 10 μM actinomycin D (a dose tolerated by the cells; was not measured). Upon normalizing the results to the control cells as in Fig. 3E, it was found that consistent with the previous reports noted above, there was no effect by transcription inhibition with actinomycin D on the up-regulation of [3H]Eb binding sites treated with nicotine relative to control values. However, TNFα enhancement of the nicotine-induced up-regulation process was abolished. In a similar experiment, inhibition of new protein synthesis with 10 μM cycloheximide was done as described above for the actinomycin D experiments. The results of these experiments, shown in Fig. 3F, demonstrate that cycloheximide abolishes essentially all up-regulation as well as TNFα enhancement of up-regulation relative to control cells. Notably, a small amount of up-regulation in cells treated with nicotine did persist, similar to that seen in membrane incubation experiments (Fig. 3C). However, we did not explicitly rule out the possibility that some residual protein synthesis could have occurred. What is important is the substantial reduction of up-regulation and enhancement by TNFα (>80% relative to controls). Together with the results of actinomycin D studies and the timing of the onset of enhanced up-regulation, these findings argue that the mechanism(s) of TNFα enhancement of [3H]Eb binding sites requires both new transcription and protein synthesis.

Up-regulation Produced by Ligands Other than Nicotine Is Enhanced by TNFα—Most agonists that bind nAChRs (e.g. nicotine) and some antagonists (e.g. dihydro-β-erythroidine) produce up-regulation (21). In this context, to examine the specificity of TNFα enhancement of up-regulation, the change in [3H]Eb binding sites by α4β2-αChR-expressing cells in the presence or absence of TNFα for 24 h and either 1 mM carbachol, 10 μM dihydro-β-erythroidine, 10 μM cytisine (a competitive partial agonist), or mecamylamine a noncompetitive antagonist not generally reported to produce up-regulation. All agents, except mecamylamine, consistently produce significant up-regulation of α4β2-nAChR [3H]Eb binding (Fig. 4). TNFα enhanced up-regulation induced by these agents proportionately to that seen with nicotine except for mecamylamine,
where again no significant effect was observed. The greatest up-regulation was observed with carbachol, although enhancement by TNFα (25 ng/ml) was proportional to that seen with other receptor ligands. Because carbachol is also an agonist of muscarinic receptors, the same experiment (Fig. 4A) was done in the presence of atropine (which by itself had no effect on [3H]Eb binding; not shown). Atropine had no measurable effect on carbachol up-regulation of [3H]Eb binding or TNFα enhancement. Also, because the concentration of carbachol used was relatively high, a dose-response assay was done (Fig. 4B). At concentrations to 60 μM, carbachol produced significant α4β2-nAChR up-regulation of [3H]Eb, and TNFα (25 ng) enhanced this up-regulation. As reported (8), the up-regulation of α4β2-nAChR by the competitive antagonist dihydro-β-erythroidine was approximately one-third that of nicotine, yet TNFα also enhanced [3H]Eb binding proportionately. Collectively, these results indicate that in all cases tested where ligand binding by the nAChR initiates the up-regulation mechanism, proportional enhancement by TNFα occurs.

Carbachol-mediated up-regulation is particularly useful, since this method permits conditions for Fura-2 calcium imaging measurements. This is because nicotine up-regulation results in receptors that are also deeply desensitized, and the extensive amounts of washing required to restore function are often impractical. In contrast, nAChR function is restored in carbachol-treated cells within 20–30 min of washing (not shown). Following 18–24 h of 500 μM carbachol, rapid application of 1 μM nicotine produces an easily measurable change in the Fura-2 340/380 ratio in ~60% of the cells (193 of 315 cells for the experiment shown; Fig. 4D), which is in contrast to con-
**TNFα Enhances Nicotinic Receptor Up-regulation**

![Graph showing the enhancement of nicotine-induced up-regulation by TNFα](image)

**FIGURE 5.** Enhancement of nicotine-induced up-regulation by TNFα (blackened portion of bar) is blocked by inhibitors of the mitogen-activated protein kinase, p38. Inhibitors of PI3K (LY294002; 100 μM) and MEK1 activation (PD98059; 30 μM) had no effect on TNFα enhancement of up-regulation (or on nicotine up-regulation alone (not shown)). In contrast, the MEK1 and MEK2 inhibitor, U0126, consistently interfered with enhanced up-regulation by ~50%. The most potent inhibitor of TNFα-enhanced up-regulation was SB 202190, a selective inhibitor of p38MAPK. Combining U0126 with SB 202190 had no additional effect. Student’s t test was used; *, p < 0.05; **, p < 0.01. NIC, nicotine.

**FIGURE 6.** Proposed model for TNFα enhancement of ligand-mediated α4β2-nAChR up-regulation. Up-regulation of α4β2-nAChR begins when nicotine (or other ligands) bind the receptor and initiates signals leading to increased receptor numbers. TNFα signaling through p38MAPK (p38) and the MEK1/2 MAPK pathways enhance this up-regulation mechanism. In this scheme, at least two pathways lead from TNFα to p38 activation. The first is through activation of p38 via the MEK1/2 and extracellular signal-regulated kinase 1/2 cascades and is partially sensitive (~50%) to inhibition of both MEK1/2 by U0126 (broken underline) but not affected by the MEK1-prefering inhibitor, PD98059. In contrast, SB 202190, a highly specific inhibitor of p38, completely blocked (as marked by solid underlines) TNFα enhancement, revealing the participation by a second pathway to activate p38. In addition to blocking p38, inhibition of transcription (actinomycin D), and protein synthesis (cycloheximide) indicate that new gene transcription and translation are required to promote enhanced up-regulation of α4β2-nAChRs.

**DISCUSSION**

Nicotine has long been associated with its ability to up-regulate the expression of α4β2-nAChRs, and this correlates with phenotypes related to addiction (1). More recently, this compound has also been found to directly impact upon inflammatory and immunologic responses (25). In a reciprocal manner, inflammatory mediators can affect the expression of nicotinic receptors (16). In this report, using stably transfected cell lines, we find that TNFα strongly enhances α4β2-nAChR up-regulation, independent of other inflammatory conditions or nAChR subtypes, through an actinomycin D and cycloheximide-sensitive p38MAPK intracellular signaling pathway. A diagram reflecting these pathways is shown in Fig. 6. The p38MAPK cascade has a central role in regulating many immunologic and inflammatory responses as well as physiological functions related to cell growth, cell differentiation, and apoptosis. Partial inhibition of TNFα-enhanced up-regulation of α4β2-nAChR by the MEK1/2 inhibitor U0126, but not the MEK1-prefering PD98059 (24), implies participation of an additional pathway that leads to p38MAPK activation. Collectively, the delay in the onset of TNFα-mediated enhancement of up-regulation, the selective diminishment of this effect by both transcription and translation inhibitors, and the general role of p38MAPK in activating stress-related genes combine to suggest that TNFα-mediated enhancement of up-regulation occurs through induction of a protein that favors nicotinic receptor expression by a...
post-translational mechanism(s). Particularly attractive candidates are chaperone-like proteins, since these would promote more efficient folding, assembly, and/or transport from the endoplasmic reticulum to the cell surface. Also, the impact of these signals appears to be based upon subunit composition. In particular, enhancement of ligand-mediated up-regulation is most notable in receptors harboring the β2 subunit. However, we observed a small but significant and direct influence by TNFα on promoting increased ligand-binding sites for α4β2-nAChR and α4β4-nAChR but not for α3-containing receptors. This indicates that the α4 subunit is also likely to harbor structures that are targeted directly by the TNFα enhancement up-regulation signal.

This study and others (13–16) also indicate that an interaction between inflammation and various nAChR subtypes occurs through multiple mechanisms. For example, α7-nAChR has been demonstrated to reduce the release of TNFα (14), which would dampen inflammation (and inflammatory cytokines), and indirectly regulate the amount of α4β2-nAChR up-regulation. However, the relationship between α7-nAChR, TNFα, and enhanced up-regulation of α4β2-nAChR may not be straightforward, since TNFα can originate from multiple sources that may or may not be regulated by α7-nAChR.

A final point is to speculate regarding the translational relevance of this observation in the animal. One place where this is possible is related to the observation that when individuals are ill, they often experience a diminished desire to continue nicotine administration. In fact this has been used as an indicator of comfort level and possibly even become toxic. A second point is related to the observation that when individuals are sick, nicotine dependence (26). Because illness is often associated with elevated TNFα, in the presence of chronic nicotine administration, enhanced up-regulation might actually exceed the comfort level and possibly even become toxic. A second point involves changes in nicotinic cholinergic receptor expression and inflammatory dysfunction that is characteristic of aging animals. There is a dramatic and selective loss of α4β2-nAChR expression in the aged brain of both rodents (27–30) and humans (especially those with Alzheimer’s disease; e.g. see Refs. 31 and 32). Coincident with the onset of old age and pathology is also an unregulated increase in TNFα and other proinflammatory cytokines. At present, the correlation between the loss of α4β2-nAChR expression and onset (or progression) of pathology is unknown. However, the possibility of a mechanistic interaction contributing to this coincidental dysregulation in both systems is intriguing. These results and other possible interactions between inflammatory cytokines and nAChR expression extend our understanding of the physiologic relevance of up-regulation as a normal contributor to local and conditional regulation of nAChR function.

REFERENCES

1. Hogg, R. C., Raggenbass, M., and Bertrand, D. (2003) Rev. Physiol. Biochim. Pharmacol. 147, 1–46
2. Schwartz, R. D., and Kellar, K. J. (1985) J. Neurochem. 45, 427–433
3. Marks, M. J., Stitzel, J. A., and Collins, A. C. (1985) J. Pharmacol. Exp. Ther. 235, 619–628
4. Benwell, M. E., Balfour, D. J., and Anderson, J. M. (1988) J. Neurochem. 50, 1243–1247
5. Flores, C. M., Rogers, S. W., Sabreza, L. A., Wolfe, B. B., and Kellar, K. J. (1992) Mol. Pharmacol. 41, 31–37
6. Zoli, M., Lena, C., Picciotto, M. R., and Changeux, J. P. (1998) J. Neurosci. 18, 4461–4472
7. Ross, S. A., Wong, Y. J., Clifford, J. J., Kinsella, A., Masalas, J. S., Horne, M. K., Scheffer, I. E., Kola, I., Waddington, J. L., Berkovic, S. F., and Drago, J. (2000) J. Neurosci. 20, 6431–6441
8. Kishi, M., and Steinbach, J. H. (2006) Mol. Pharmacol. 70, 2037–2044
9. Vallejo, Y. F., Buisson, B., Bertrand, D., and Green, W. N. (2005) J. Neurosci. 25, 5563–5572
10. Lukas, R. J., Changeux, J. P., Le Novere, N., Albuquerque, E. X., Balfour, D. J., Berg, D. K., Bertrand, D., Chiappinelli, V. A., Clarke, P. B., Collins, A. C., Dani, J. A., Grady, S. R., Kellar, K. J., Lindstrom, J. M., Marks, M. J., Quik, M., Taylor, P. W., and Wonnacott, S. (1999) Pharmacol. Rev. 51, 1–401
11. Wu, J., Liu, Q., Yu, K., Hu, J., Kuo, Y. P., Segerberg, M., St John, P. A., and Lukas, R. J. (2006) J. Physiol. 576, 103–118
12. Xiao, Y., Baydruk, M., Wang, H. P., Davis, H. E., and Kellar, K. J. (2004) Bioorg. Med. Chem. Lett. 14, 1845–1848
13. Carlson, N. G., Bacchi, A., Rogers, S. W., and Gahring, L. C. (1998) J. Neurobiol. 35, 29–36
14. Wang, H., Yu, M., Ochani, M., Amella, C. A., Tanovic, M., S公交车, Li, J. H., Yang, H., Ulloa, L., Al-Abed, Y., Czura, C. J., and Tracey, K. J. (2003) Nature 421, 384–388
15. Carlson, N. G., Wieggel, W. A., Chen, J., Bacchi, A., Rogers, S. W., and Gahring, L. C. (1999) J. Immunol. 163, 3963–3968
16. Gahring, L. C., Days, E. L., Kaasch, T., Gonzalez de Mendoza, M., Owen, L., Persiyanov, K., and Rogers, S. W. (2005) J. Neuroimmunol. 166, 88–101
17. Orr-Urtreger, A., Kedmi, M., Rosner, S., Karmeli, F., and Rachmilewitz, D. (2005) Neuroreport 16, 1123–1127
18. Xiao, Y., Meyer, E. L., Thompson, J. M., Surin, A., Wroblewski, J., and Kellar, K. J. (1998) Mol. Pharmacol. 54, 322–333
19. Xiao, Y., and Kellar, K. J. (2004) J. Pharmacol. Exp. Ther. 310, 98–107
20. Rogers, S. W., Gregori, N. Z., Carlson, N., Gahring, L. C., and Noble, M. (2001) Glia 33, 306–313
21. Gopalakrishnan, M., Molinari, E. J., and Sullivan, J. P. (1997) Mol. Pharmacol. 52, 524–534
22. MacEwan, D. J. (2002) Cell. Signal. 14, 477–492
23. Hommes, D. W., Peppelenbosch, M. P., and van Deventer, S. J. (2003) Gut 52, 144–151
24. Karihaloo, A., O’Rourke, D. A., Nickel, C., Spokes, K., and Cantley, L. G. (2001) J. Biol. Chem. 276, 9166–9173
25. Wang, H., Liao, H., Ochani, M., Ulloa, L., Al-Abed, Y., Yang, H., Metz, C., Miller, E. J., and Tracey, K. J. (2004) Nat. Med. 10, 1216–1221
26. Heatherton, T. F., Kozlowski, L. T., Freerker, R. C., and Fagerstrom, K. O. (1991) J. Addict. 86, 1119–1127
27. Schulz, D. W., Kuchel, G. A., and Zigmund, R. E. (1993) J. Neurochem. 61, 2225–2232
28. Rogers, S. W., Gahring, L. C., Collins, A. C., and Marks, M. (1998) J. Neurosci. 18, 4825–4832
29. Perry, D. C., Xiao, Y., Nguyen, H. N., Musachio, J. L., Davila-Garcia, M. I., and Kellar, K. J. (2002) J. Neurochem. 82, 468–481
30. Gahring, L. C., Persiyanov, K., and Rogers, S. W. (2005) Neurobiol. Aging 26, 973–980
31. Kellar, K. J., Whitehouse, P. J., Martino-Barrows, A. M., Marcus, K., and Price, D. L. (1987) Brain Res. 436, 62–68
32. Schroder, H., Giacobini, E., Struble, R. G., Zilles, K., Maelicke, A., Luiten, P. G., and Strosberg, A. D. (1991) Ann. N. Y. Acad. Sci. 640, 189–192