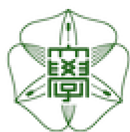
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1 **Alpha and beta adrenoceptors activate interleukin-6 transcription through different**

2 **pathways in cultured astrocytes from rat spinal cord**

3

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14

Abstract

In brain astrocytes, noradrenaline (NA) has been shown to up-regulate IL-6 production via β-adrenoceptors (ARs). However, the underlying intracellular mechanisms for this regulation is not clear, and it remains unknown whether α-ARs are involved. In this study, we investigated the AR-mediated regulation of IL-6 mRNA levels in the cultured astrocytes from rat spinal cord. NA, the α_1-agonist phenylephrine, and the β-agonist isoproterenol increased IL-6 mRNA levels. The phenylephrine-induced IL-6 increase was accompanied by an increase in ERK phosphorylation, and these effects were blocked by inhibitors of PKC and ERK. The isoproterenol-induced IL-6 increase was accompanied by an increase in CREB phosphorylation, and these effects were blocked by a PKA inhibitor. Our results indicate that IL-6 increases by α_1- and β-ARs are mediated via the PKC/ERK and cAMP/PKA/CREB pathways, respectively. Moreover, conditioned medium collected from astrocytes treated with the α_2-AR agonist dexmedetomidine, increased IL-6 mRNA in other astrocytes. In this study, we elucidate that α_1- and α_2-ARs, in addition to β-ARs, promote IL-6 transcription through different pathways in spinal cord astrocytes.

Keywords

astrocyte; noradrenaline; adrenoceptor; interleukin-6; MAPK

1. Introduction

Noradrenergic neurons project to most of regions of the central nervous system (CNS) [1]. Levels of noradrenaline (NA) in the CNS change under physiological and pathological conditions, such as ischemic stroke, neuropathic pain, and Alzheimer's disease [2–4]. Furthermore, inactivation of noradrenergic neurons enhances inflammation [5] and conversely, promotes recovery from ischemic brain injury [6], suggesting that NA is involved in the pathogenesis of the CNS diseases.

In the CNS, NA is released not only from synapses, but also from varicosities [7], and thus acts on astrocytes surrounding neurons. NA up-regulates the production of neurotrophins, growth factors, and proinflammatory cytokines such as interleukin-6 (IL-6) in astrocytes [8,9]. IL-6 has various roles besides enhancing inflammation, acts as a neurotrophic factor [10], and suppresses neuronal cell death [9]. Since the major source of IL-6 in the CNS is astrocytes [11], NA could affect physiological functions and disease pathogenesis by modulating IL-6 production via astrocytic adrenoceptors (ARs). There are two main groups of ARs, i.e., α and β, with 9 subtypes in total [12]; astrocytes express α_1-, α_2- and β-ARs [13]. In cultured cerebral astrocytes, NA increases IL-6 production via activation of β-ARs [14]. However, the intracellular mechanisms that underlie NA-induced IL-6 production in astrocytes are not fully explained. In addition, it is not clear whether α-ARs are involved in IL-6 production. It was reported that α-ARs were not involved in

54 NA-activated IL-6 transcription in astrocytes [9], conversely, another study showed that
55 NA-activated IL-6 production was inhibited by an α-AR antagonist [15]. In both studies,
56 only antagonists rather than both agonists and antagonists were used, and the expression
57 of AR-subtypes was not confirmed. Astrocytic α-ARs have functionally significant roles,
58 such as regulation of neuronal activity and blood flow [16–18]. Since these functions may
59 be related to astrocytic IL-6 production, it is important to elucidate the involvement of α-
60 ARs in IL-6 production.

61 In the spinal cord, the concentration of NA changes in response to ischemia,
62 neuropathic pain, and aging [4,19,20]. *In vivo* administration of IL-6 promotes axonal
63 sprouting and synapse formation, leading regeneration and functional recovery after
64 spinal cord injury [21]. Furthermore, astrocytes show regional differences in receptor
65 expression, transcriptional factor activity, and responses to inflammation [22–24].
66 Therefore, it is worth investigating the contribution of ARs to the regulation of IL-6 in
67 spinal cord astrocytes.

68 In this study, we identified AR-subtypes that participate in the transcriptional
69 regulation of IL-6 and investigated the intracellular signaling following the activation of
70 each AR-subtypes in the cultured astrocytes from rat spinal cord.
71

72 **2. Materials and methods**

73

74 **2.1. Materials**

75 Antibodies against ERK1/2 (#4695S, 1:2500), phospho-ERK 1/2 (#9101S, 1:2500),
76 p38 (#9212S, 1:2000), phospho-p38 (#9211S, 1:1000), SAPK/JNK (#9252S, 1:2500),
77 phospho-SAPK/JNK (#9251S, 1:1500), STAT3 (#4904S, 1:4000), and phospho-STAT3
78 (#9145S, 1:2000) were purchased from Cell Signaling Technology (Danvers, MA, USA).
79 Antibody against CREB (#sc-377154, 1:500) and phospho-CREB (#sc-81486, 1:250) were
80 purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A peroxidase-
81 conjugated mouse anti-glyceraldehyde 3-phosphate dehydrogenase antibody (GAPDH;
82 #G9295, 1:50000), dexmedetomidine hydrochloride, atipamezole hydrochloride, and
83 isoproterenol hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA).
84 Phenylephrine hydrochloride, propranolol hydrochloride, forskolin, U0126, and SP600125
85 were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan). L-noradrenaline
86 bitartrate monohydrate and prazosin hydrochloride were purchased from Tokyo Chemical
87 Industry (Tokyo, Japan). H89 and bisindolylmaleimide II (BIM) were purchased from
88 Cayman Chemical (Ann Arbor, MI, USA).

89

90 **2.2. Animals**

91 All animal care and experimental protocols were approved by the Committee on
92 Animal Experimentation, Graduate School of Veterinary Medicine, Hokkaido University
93 (No. 19-0009), which has been awarded Accreditation Status by the Association for
94 Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.
95 Wistar rats were obtained from CLEA Japan (Tokyo, Japan). Male and female pups aged
96 3–5 days were used for primary astrocyte cultures.

97

98 **2.3. Primary cultures of spinal cord astrocytes**

99 Primary cultures of spinal cord astrocytes were obtained as previously described
100 [25]. In brief, spinal cords were isolated from rat pups, minced, and incubated with papain
101 (10 U/ml) and DNase (0.1 mg/ml). Dissociated cells were suspended in Dulbecco's Modified
102 Eagle's Medium/Ham's F-12 containing 10% fetal bovine serum, 100 U/ml penicillin, and
103 0.1 mg/ml streptomycin. The cell suspension was seeded onto a poly-l-lysine-coated T75
104 flask. After 7–8 days, the flask was shaken at 250 rpm at 37°C for at least 12 h to remove
105 all cells except for astrocytes. Adherent cells were detached with trypsin and re-seeded
106 onto poly-l-lysine-coated 6- and 12-well plates at a density of 8.0×10^3 cells/cm^2. After 3
107 days, the cell culture had reached confluence and the medium was changed to serum-free
108 medium. Cell cultures were treated with NA or AR-agonists 1 h after the medium

109 exchange, and were treated with AR-antagonists or inhibitors immediately after the

110 exchange. After the given time (detailed in the figure legends and results section), the cell

111 culture was used for experiments.

112

113 **2.4. RNA extraction and real-time PCR analyses**

114 Total RNAs were extracted from cultured astrocytes using RNAiso Plus (Takara

115 Bio, Tokyo, Japan). To remove genomic DNA and synthesize cDNA, the RNA sample was

116 then incubated with qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka, Japan).

117 Real-time PCR was performed using Thunderbird SYBR qPCR Mix (TOYOBO), each

118 primer, and the cDNA reaction solution. The primers used were as follows: 5'- GAT TGT

119 ATG AAC AGC GAT GAT GC-3' (forward) and 5'- AGA AAC GGA ACT CCA GAA GAC C -

120 3' (reverse) for IL-6; 5'- GCA AGA GAG AGG CCC TCA G -3' (forward) and 5'- TGT GAG

121 GGA GAT GCT CAG TG -3' (reverse) for GAPDH. Thermal cycles were performed using

122 Eco Real Time PCR System (Illumina, CA, USA). Cycling conditions were 95°C for 1 min

123 (for initial denaturation), followed by 40 cycles of denaturation (95°C, 15 s), annealing and

124 extension (61°C, 45 s). RNAs without reverse transcription were used as a negative control

125 to examine DNA contamination and these were not amplified by real-time PCR. Melt curve

126 analysis confirmed that the obtained amplicon was only the one expected in each reaction.

127 The expression levels of the IL-6 relative to GAPDH were calculated by the ΔΔCq method

128 and were expressed as relative to the control, which was arbitrarily set to a value of "1.0".

129

130 **2.5. Western blotting**

131 Astrocytes were lysed in RIPA buffer containing a protease inhibitor cocktail

132 (nacalai tesque, Kyoto, Japan). The samples were separated by 10% SDS-PAGE and

133 transferred to polyvinylidene difluoride membranes (Millipore, CA, USA). The membranes

134 were blocked with 5% skimmed milk and then incubated with a primary antibody at 4°C

135 for at least 12 h. Thereafter, the membranes were incubated for 1 h at room temperature

136 with a horseradish peroxidase-conjugated secondary antibody (GE Healthcare, Little

137 Chalfont, UK). Antibody binding was visualized by ECL Prime (GE Healthcare). Band

138 intensities were measured using ImageJ software (National Institutes of Health) and

139 expressed as relative to the control, which was arbitrarily set to a value of "1.0".

140

141 **2.6. Preparation and treatments of astrocyte conditioned medium**

142 Agonists or antagonists were treated to the primary cultures of astrocytes in the

143 manner described above. Following agonist treatments for 30 min, cells were refreshed

144 with serum-free medium. After 24 h incubation, the medium was collected from each

145 treated well. RNA extraction was performed at the same time as the medium was collected.

146 The medium was centrifuged at 300 × g to remove dead cells and the supernatant was

147 used as astrocyte conditioned medium (ACM). The drug-treated astrocytes to produce
148 ACM were defined as ACM-donor cells. The ACM was transferred to another primary
149 culture of astrocytes, which was defined as ACM-recipient cells. The ACM-recipient cells
150 were incubated for 3 h followed by RNA extraction.
151
152 **2.7. Data analysis**
153 Data are expressed as means ± S.E.M (n = number of independent measurements).
154 Statistical comparisons between two groups were made using the unpaired Student's t-
155 test. For multiple comparisons, one-way ANOVA followed by the Dunnett's test was used.
156 A value of $p < 0.05$ was considered as a statistically significant level. All statistical analysis
157 was performed with Ekuseru-Toukei 2008 (Social Survey Research Information Co., Ltd.,
158 Tokyo, Japan).
159

160 **3. Results**

161

162 **3.1. Effects of AR-agonists and antagonists on IL-6 mRNA levels**

163 We have previously confirmed the expression of α_1-, α_2- and β-ARs by RT-PCR
164 analysis in the cultured astrocytes from rat spinal cord [26]. Treatment of astrocytes with
165 NA (1 or 10 μM) increased the IL-6 mRNA level (Fig. 1A), which reached a peak 1 h after
166 treatment and remained significantly higher than the untreated control for up to 6 h. The
167 treatment of either NA or the β-agonist isoproterenol for 3 h increased IL-6 mRNA level in
168 a concentration-dependent manner (Fig. 1B). The maximum increase in the IL-6 mRNA
169 level was higher in response to NA than that it was to isoproterenol.

170 In addition to isoproterenol (1 μM), the IL-6 mRNA level was increased by the α_1-
171 agonist phenylephrine (1 μM) but not by the α_2-agonist dexmedetomidine (1 μM) (Fig. 1C).
172 The NA-induced increase in the IL-6 mRNA level was inhibited by the α_1-antagonist
173 prazosin (1 μM), the α_2-antagonist atipamezole (10 μM), and the β-antagonist propranolol
174 (10 μM) (Fig. 1D). Co-treatment with prazosin and propranolol abolished the NA-induced
175 increase in the IL-6 mRNA level. Additional treatment with atipamezole did not cause
176 further inhibition. None of the antagonists alone had any effect on IL-6 mRNA levels (Fig.
177 1E).

178

179 **3.2. Mechanisms of increase in IL-6 mRNA via β-ARs**

180 In general, β-ARs coupled to Gs proteins activate the cAMP/protein kinase A
181 (PKA) pathway. The isoproterenol-induced increase in the IL-6 mRNA level was inhibited
182 by the PKA inhibitor H89 (5 μM), but not by the protein kinase C (PKC) inhibitor BIM (5
183 μM) (Fig. 2A). The adenylyl cyclase activator forskolin (10 μM) also increased the IL-6
184 mRNA level (Fig. 2B). PKA activates cAMP response element binding protein (CREB),
185 which regulates the expression of a variety of genes [27]. Isoproterenol increased CREB
186 phosphorylation 30 min after treatment (Fig. 2C). In the presence of propranolol or H89,
187 isoproterenol did not increase CREB phosphorylation. Forskolin also increased CREB
188 phosphorylation. These results suggest that the cAMP/PKA/CREB pathway is involved in
189 the increase in IL-6 mRNA level following β-AR activation.

190

191 **3.3. Mechanisms of increase in IL-6 mRNA via α_1-ARs**

192 α_1-ARs coupled to Gq proteins activate the Ca^{2+}/PKC pathway, which increases
193 mitogen-activated protein kinases (MAPKs) phosphorylation [28]. Therefore, we
194 investigated whether PKC and/or MAPKs participates in the transcriptional activation of
195 IL-6 following α_1-AR activation. The phenylephrine-induced increase in the IL-6 mRNA
196 level was inhibited by BIM, but not by H89 (Fig. 3A). Phenylephrine did not affect CREB
197 phosphorylation (Fig. 3B). The MAPK-ERK (extracellular signal-regulated kinase) kinase

198 (MEK)/ERK inhibitor U0126 (10 μM) abolished the phenylephrine-induced increase in the

199 IL-6 mRNA level (Fig. 3C). Phenylephrine increased ERK phosphorylation (Fig. 3D). In

200 the presence of prazosin, BIM, or U0126, phenylephrine did not increase ERK

201 phosphorylation. On the other hand, phenylephrine did not affect c-jun N-terminal kinase

202 (JNK) or p38 phosphorylation (Fig. 3E, F). These results suggest that the PKC/ERK

203 pathway is involved in the increase in IL-6 mRNA level following α_1-AR activation.

204

205 **3.4. Effects of MAPKs or PKA inhibitor on NA-induced transcriptional activation of IL-6**

206 NA increased ERK and JNK phosphorylation but not p38, and these increases

207 were inhibited by prazosin (Fig. 3D, E, F). Therefore, we investigated whether ERK or

208 JNK phosphorylation affects the NA-induced increase in the IL-6 mRNA levels. U0126

209 and the JNK inhibitor SP600125 (10 μM) suppressed the NA-induced ERK and JNK

210 phosphorylation (Fig. 4A, B). Furthermore, U0126 and SP600125 abolished the NA-

211 induced IL-6 increase (Fig. 4C). SP600125 alone did not have any effect on IL-6 mRNA

212 level (Fig. 4D). Moreover, H89 abolished the NA-induced IL-6 increase (Fig. 4E). Since IL-

213 6 transcription is activated by STAT3 phosphorylation [29], we investigated the effect of

214 AR-agonists on it. NA, phenylephrine, and isoproterenol had no effect on STAT3

215 phosphorylation (Fig. 4F). These results suggest that both cAMP/PKA and ERK and/or

216 JNK pathways are involved in the NA-induced increase in the IL-6 mRNA level.

217

218 **3.5. Effects of AR-agonists on IL-6 mRNA levels via astrocyte conditioned medium.**

219 We tested the long-term or indirect effects on IL-6 transcription following AR-
220 activation. The treatment of NA or AR-agonists for 24 h did not have any effect on IL-6
221 mRNA levels (Fig. 5A). Then, we examined whether some factors released by AR-
222 activation are involved in IL-6 transcription. The 3 h treatment of astrocyte conditioned
223 medium (ACM), derived from the ACM-donor cells treated with NA or dexmedetomidine
224 but not phenylephrine and isoproterenol, increased IL-6 mRNA levels in the ACM-
225 recipient cells (Fig. 5B, C). The effect of dexmedetomidine was abolished by the treatment
226 of atipamezole for the ACM-donor cells (Fig 5D). However, there were no changes in IL-6
227 mRNA levels in the ACM-donor cells (Fig. 5E). These results suggest that α_2-ARs increase
228 IL-6 mRNA level via some factors released into the extracellular medium.
229

230 **4. Discussion**

231 In this study, we elucidate that α_1- and α_2-ARs, in addition to β-ARs, are involved
232 in the transcriptional activation of IL-6 in astrocytes. Moreover, we showed that AR-
233 mediated activation of IL-6 transcription involves CREB and MAPKs. Astrocytic ARs are
234 likely to play a role in physiological and/or pathophysiological functions in the CNS by
235 regulating the production of IL-6.

236 NA activated the transcription of IL-6 in spinal cord astrocytes. In this study, NA
237 concentration-dependently increased IL-6 transcription at concentrations of 10 nM to 1
238 μM. The physiological concentration of NA in cerebrospinal fluid is 1 nM to 100 nM [2,30].
239 Therefore, physiological concentrations of NA are sufficient to induce IL-6 production by
240 astrocytes. In addition, since NA levels increase in ischemia and neuropathic pain [2,4]
241 and decrease in Alzheimer's disease [3], IL-6 production by NA in astrocytes is likely to
242 change under pathological conditions.

243 The maximum effect of NA on IL-6 mRNA transcription was higher than that of
244 isoproterenol. NA has a higher affinity for α-ARs than β-ARs [31]. The effect of NA on IL-
245 6 transcription was suppressed by both α_1- and β-antagonists, and the α_1-agonist
246 phenylephrine also activated the IL-6 transcription. These results indicate that α_1-ARs
247 are involved in the transcriptional activation of IL-6 in spinal cord astrocytes. On the other
248 hand, in cultured cerebral astrocytes, NA activates IL-6 transcription, which is inhibited

249 by antagonists for β-ARs, but not for α-ARs [9]. Moreover, α_1-AR activation stimulates

250 ERK phosphorylation in cultured spinal cord astrocytes [32], but not in cerebral astrocytes

251 [33]. Astrocytes show regional differences in receptor expression, transcriptional factor

252 activity, and responses to inflammation [22–24]. Therefore, these astrocyte heterogeneities

253 may be involved in the discrepancy in the involvement of α_1-ARs between spinal and

254 cerebral astrocytes.

255 The α_2-antagonist atipamezole suppressed the effect of NA on IL-6, whereas the

256 treatment of the α_2-agonist dexmedetomidine for 3 h showed no effect. Furthermore, the

257 effect of NA on IL-6 transcription was completely suppressed by co-treatment with α_1- and

258 β-AR antagonists, indicating that NA activates IL-6 transcription mainly via α_1- and β-

259 ARs. One of the possible explanations for this discrepancy between the agonist's and

260 antagonist's effects is that the mechanisms involved in the activation of one receptor

261 subtype by a specific agonist are different from the mechanisms involved in the activation

262 of multiple receptor subtypes by NA. For example, β_1- and α_{2A}, or α_{1A}- and α_{1B}-ARs form

263 receptor-heterodimers [34,35]. When only one receptor from the heterodimer is activated,

264 the intracellular signaling of the heterodimer is different to the signaling seen when both

265 receptors in the heterodimer are activated [36,37]. This could provide an explanation for

266 why NA-increased JNK phosphorylation was suppressed by prazosin, while exerting no

267 effect of phenylephrine on the JNK phosphorylation. Further investigations are needed to

268 reveal these mechanisms.

269 Transcription of IL-6 is promoted by binding of transcription factors, such as AP1,
270 CREB, and NF-κB, to specific DNA sequences and p300/CBP [38,39]. In addition,
271 activation of MAPKs signaling can lead to AP1 activation [40]. In this study,
272 phenylephrine activated ERK and IL-6 transcription, both of which were suppressed by
273 the PKC and ERK inhibitor. Phenylephrine also did not affect CREB, JNK, and p38
274 phosphorylation. These results indicate that α_1-ARs activate IL-6 transcription via the
275 PKC/ERK pathway in spinal cord astrocytes. Meanwhile, isoproterenol activated CREB
276 and IL-6 transcription, both of which were suppressed by the PKA inhibitor. The adenylate
277 cyclase activator forskolin mimicked the action of isoproterenol. These results indicate
278 that β-ARs activate IL-6 transcription via the cAMP/PKA/CREB pathway in spinal cord
279 astrocytes. As in this study, the effects of β-ARs on IL-6 secretion or transcription are
280 mediated via the cAMP/PKA pathway in cerebral astrocytes [9,14]. Moreover, the NA-
281 activated transcription of IL-6 was inhibited by both the ERK inhibitor and the JNK
282 inhibitor. The phosphorylation of ERK or JNK activates common factors, such as c-jun and
283 Elk [41], which may be also involved in IL-6 transcription. NA-activated transcription of
284 IL-6 was also inhibited by the PKA inhibitor. Therefore, the pathway of NA-activated IL-
285 6 transcription is likely to include the pathways activated by phenylephrine and
286 isoproterenol. In addition to these pathways, STAT3 phosphorylation activates IL-6

287 transcription in astrocytes [29], but NA, phenylephrine, and isoproterenol had no effect on

288 STAT3 phosphorylation in this study. Therefore, the STAT3 pathway is likely not involved

289 in AR-induced IL-6 transcriptional activation in astrocytes. Furthermore, in contrast to

290 this study, it has been shown that isoproterenol suppresses lipopolysaccharide (LPS)-

291 induced IL-6 promoter activities via β_2-ARs in astrocytes [42]. LPS-induced IL-6

292 transcriptional activation is mediated via the TLR4-NF-κB pathway [43,44], and the

293 activation of β-ARs attenuates NF-κB activity by increasing IκBα gene expression and

294 protein levels [45]. In the presence of LPS, β-ARs may suppress IL-6 transcription by

295 potent suppressing NF-κB. Therefore, it is likely that the activation of β-ARs shows

296 bidirectional effects on IL-6 transcription via the CREB and NF-κB pathways.

297 ACM derived from NA- or dexmedetomidine-treated astrocytes increased IL-6

298 mRNA levels in the ACM-recipient cells. It is possible that factor(s), released into the

299 extracellular medium by activation of α_2-ARs, may promote IL-6 transcription in

300 neighboring and distant astrocytes. Activation of astrocytic α_2-ARs increases

301 accumulation of glutamine, as a precursor of neurotoxic glutamate [46], and activation of

302 metabotropic glutamate receptors enhances the release or transcription of IL-6 in

303 astrocytes [47,48]. In addition, the effect of released factor(s) may disappear within a few

304 hours, because there were no changes in IL-6 mRNA levels in the ACM-donor cells.

305 Further studies are needed to elucidate the indirect pathway of IL-6 transcriptional

306 activation via the α_2-ARs.

307 Intraperitoneal injection of lipopolysaccharide to mice increases IL-6 mRNA levels
308 several-fold in brain astrocytes *in vivo* [49]. The IL-6 increases in responses to NA or AR-
309 agonists in this study were similar in magnitude to these *in vivo* studies. Besides immune
310 responses in the CNS, IL-6 acts as a neurotrophic factor [10], induces the differentiation
311 of neural stem cells [50], and promotes vasculogenesis during the brain development [51].
312 Therefore, under physiological conditions, NA may play a beneficial role in the CNS
313 functions by promoting IL-6 release from astrocytes. Conversely, treatment of spinal cord-
314 injured rats with anti-IL-6 neutralizing antibodies reduces the area of injury [52]. Under
315 pathological conditions, NA may act on astrocytes to promote IL-6 production and delay
316 recovery. Further studies are needed to reveal whether astrocytic ARs regulate IL-6
317 production *in vivo* and how astrocytic IL-6 acts under physiological and pathological
318 conditions.
319
320 **5. Conclusion**

321 In this study, we elucidated that NA promotes the transcriptional activation of IL-
322 6 in spinal cord astrocytes, and α_1- and α_2-ARs, in addition to β-ARs, are involved. IL-6
323 increase by α_1- and β-ARs are mediated via the PKC/ERK and cAMP/PKA/CREB pathways,
324 respectively. Moreover, astrocyte conditioned medium collected from cells treated with the
325 α_2-AR agonist dexmedetomidine, increased IL-6 mRNA in other astrocytes. Based on these

326 results, we suggest a role for NA in the CNS homeostasis and pathogenesis via modulation

327 of cytokine production by astrocytic ARs.

328

329 **CRediT authorship contribution statement**

330 **Kohei Morimoto:** Conceptualization, Data curation, Formal analysis,
331 Investigation, Visualization, Writing – original draft. **Ryota Eguchi:** Funding acquisition,
332 Writing - review & editing. **Taisuke Kitano:** Investigation, Writing - review & editing. **Ken-**
333 **ich Otsuguro:** Conceptualization, Supervision, Funding acquisition, Writing - review &
334 editing.

335

336 **Declaration of competing interest**

337 The authors declare that they have no known competing financial interests or
338 personal relationships that could have appeared to influence the work reported in this
339 paper.

340

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345

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534

535 **Figure legends**

536

537 **Figure 1. Effects of NA, AR-agonists, and AR-antagonists on IL-6 mRNA levels in cultured**

538 **astrocytes**

539 (A) IL-6 mRNA levels in astrocytes treated with NA (1 and 10 μM) for 1, 3, 6, and 12 h. $*p$

540 < 0.05, $**p < 0.01$ vs. time-matched control (Dunnett's test), n = 6. (B) IL-6 mRNA levels

541 in astrocytes treated with NA or the β-agonist isoproterenol (ISO) (1 nM-10 μM) for 3 h.

542 $*p < 0.05$, $**p < 0.01$ vs. control (Dunnett's test), n = 6. (C) IL-6 mRNA levels in astrocytes

543 treated with the α_1-agonist phenylephrine (PHE, 1 μM), the α_2-agonist dexmedetomidine

544 (DEX, 1 μM), and ISO (1 μM) for 3 h. $**p < 0.01$ vs. control (Dunnett's test), n = 6. (D) IL-

545 6 mRNA levels in astrocytes treated with NA (1 μM) in the presence or absence of the α_1-

546 antagonist prazosin (PRAZ, 1 μM), the α_2-antagonist atipamezole (ATIP, 10 μM), and the

547 β-antagonist propranolol (PROP, 10 μM) for 3 h. $##p < 0.01$ vs. NA alone (Dunnett's test),

548 n = 6. (E) IL-6 mRNA levels in astrocytes treated with PRAZ, ATIP, and PROP for 4 h. n =

549 6. Data are presented as means ± S.E.M. The mRNA levels of cytokines were expressed as

550 relative to the control, which was arbitrarily set to a value of "1.0".

551

552 **Figure 2. Intracellular mechanisms of transcriptional activation of IL-6 via β-ARs**

553 (A, B) IL-6 mRNA levels in astrocytes treated with the β-agonist isoproterenol (1 μM), the

554 adenylyl cyclase activator forskolin (FSK, 10 μM) in the presence or absence of the PKA
555 inhibitor H89 (5 μM) and the PKC inhibitor BIM (5 μM) for 3 h. #$p < 0.05$ (Dunnett's test),
556 **$p < 0.01$ (unpaired Student's t-test), n.s.: not significant, $n = 6$. (C) The protein expression
557 levels of phosphorylated and total CREB were quantified and representative blots are
558 shown. GAPDH was used as a loading control. Astrocytes were treated with isoproterenol
559 and FSK in the presence or absence of the β-antagonist propranolol (PROP, 10 μM) and
560 H89 for 30 min. **$p < 0.01$ vs. control (Dunnett's test), $n = 6$. Data are presented as means
561 ± S.E.M.
562

563 **Figure 3. Intracellular mechanisms of transcriptional activation of IL-6 via α_1-ARs**

564 (A, C) IL-6 mRNA levels in astrocytes treated with the α_1-agonist phenylephrine (1 μM) in
565 the presence or absence of the PKC inhibitor BIM (5 μM), the PKA inhibitor H89 (5 μM),
566 and the MEK/ERK inhibitor U0126 (10 μM) for 3 h. ##$p < 0.01$ (Dunnett's test), **$p < 0.01$
567 (unpaired Student's t-test), n.s.: not significant, $n = 6$. (B, D-F) The protein expression
568 levels of phosphorylated and total CREB (B), ERK (D), JNK (E), and p38 (F) were
569 quantified and representative blots are shown. GAPDH was used as a loading control.
570 Astrocytes were treated with phenylephrine (PHE), or NA (1 μM) in the presence or
571 absence of the α_1-antagonist prazosin (PRAZ, 1 μM), BIM and U0126 for 30 min (B) or 10
572 min (D-F). *$p < 0.05$, **$p < 0.01$ vs. control (Dunnett's test), $n = 6$. Data are presented as

573 means ± S.E.M.

574

575 **Figure 4. Effects of ERK, JNK, and PKA inhibitor on NA-induced transcriptional**
576 **activation of IL-6.**

577 (A, B, F) The protein expression levels of phosphorylated and total ERK (A), JNK (B), and
578 STAT3 (F) were quantified and representative blots are shown. GAPDH was used as a
579 loading control. Astrocytes were treated with NA (1 μM), the α_1-agonist phenylephrine
580 (PHE, 1 μM), and the β-agonist isoproterenol (ISO, 1 μM) in the presence or absence of the
581 MEK/ERK inhibitor U0126 (10 μM) and the JNK inhibitor SP600125 (SP, 10 μM) for 10
582 min (A, B) or 30 min (F). #$p < 0.05$, ##$p < 0.01$ vs. NA alone (Dunnett's test), n = 6. (C, E)
583 IL-6 mRNA levels in astrocytes treated with NA in the presence or absence of U0126, SP
584 and the PKA inhibitor H89 (5 μM) for 3 h. ##$p < 0.01$ vs. NA alone (Dunnett's test), n = 6.
585 (D) IL-6 mRNA levels in astrocytes treated with SP600125 for 4 h. n = 6. Data are
586 presented as means ± S.E.M.

587

588 **Figure 5. Effects of AR-agonists on IL-6 mRNA levels via astrocyte conditioned medium.**

589 (A) IL-6 mRNA levels in astrocytes treated with NA (1 μM), the α_1-agonist phenylephrine
590 (PHE, 1 μM), the α_2-agonist dexmedetomidine (DEX, 1μM), and the β-agonist isoproterenol
591 (1 μM) for 24 h, n = 6. (B) Schematic depiction of astrocyte conditioned medium (ACM)

592 transfer experiment. (C, D) IL-6 mRNA levels in astrocytes incubated with ACM derived

593 from astrocytes treated with NA, PHE, DEX and ISO in the presence or absence of α_2-

594 antagonist atipamezole (ATIP, 1 μM) for 3 h. *$p < 0.05$, **$p < 0.01$ vs. control (C, Dunnett's

595 test), #$p < 0.05$, ##$p < 0.01$ vs. DEX alone (D, Dunnett's test), n = 6. (E) IL-6 mRNA levels

596 in astrocytes treated with DEX in the presence or absence of ATIP for 30 min and

597 incubated for 24 h following medium exchange. n = 6. Data are presented as means ±

598 S.E.M.

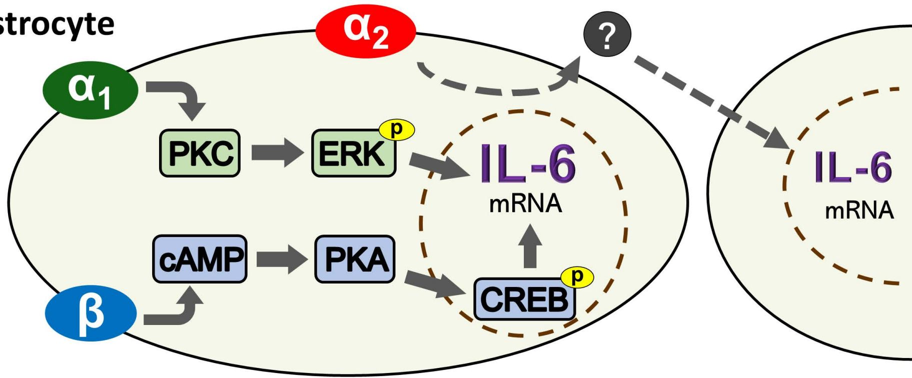
Astrocyte
α2
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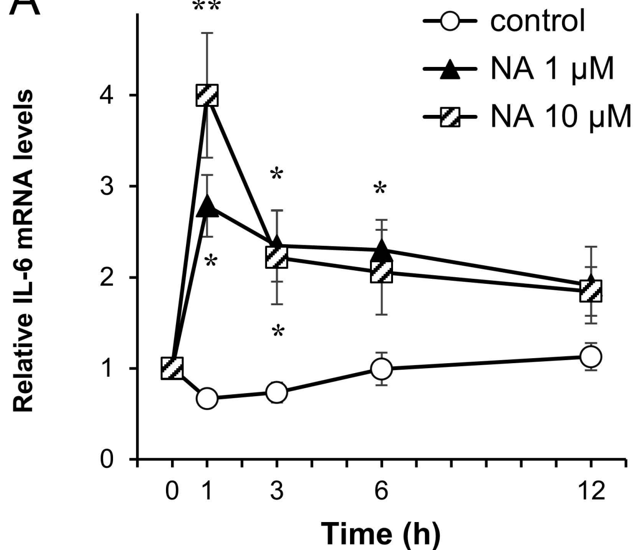
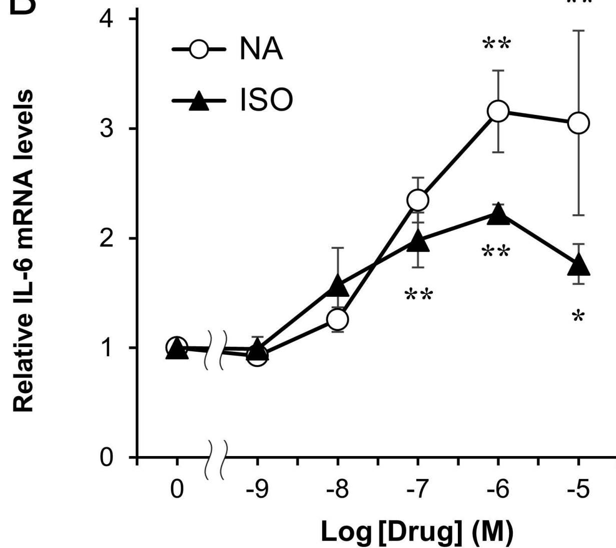
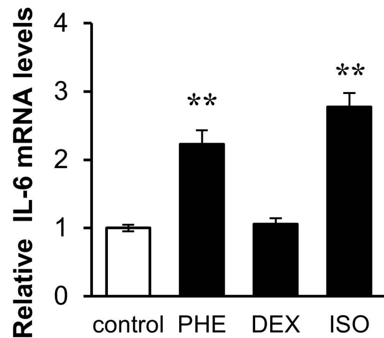
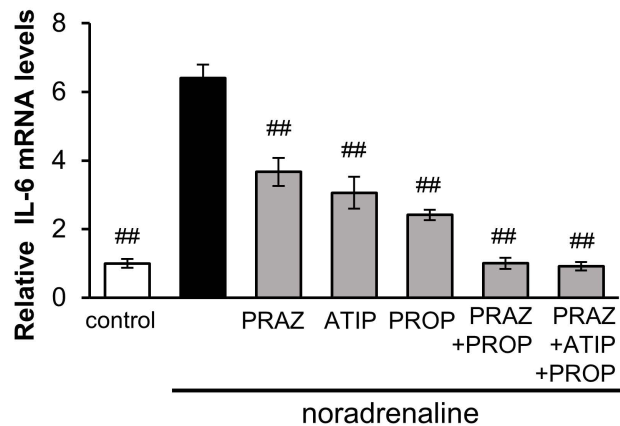
Fig.1









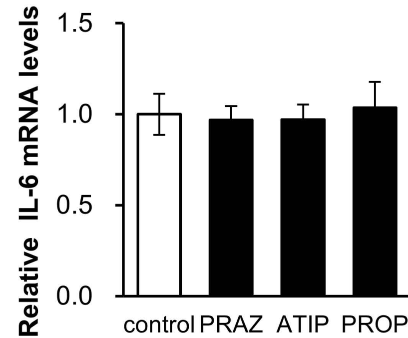
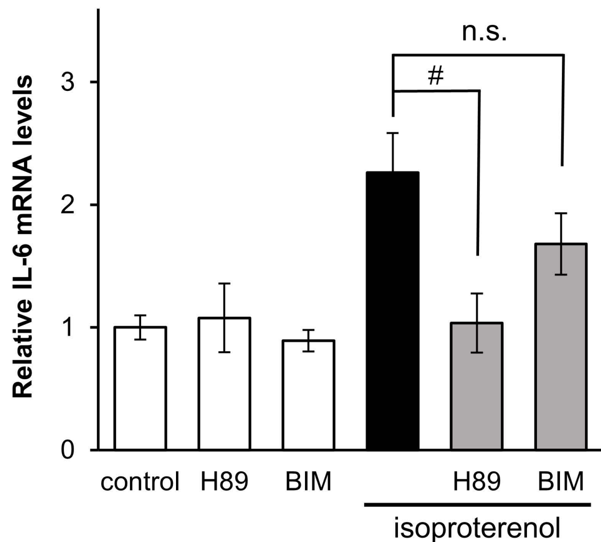
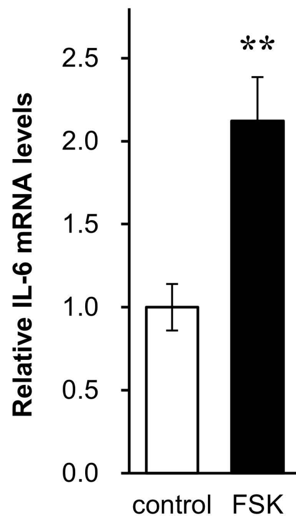


Fig. 2





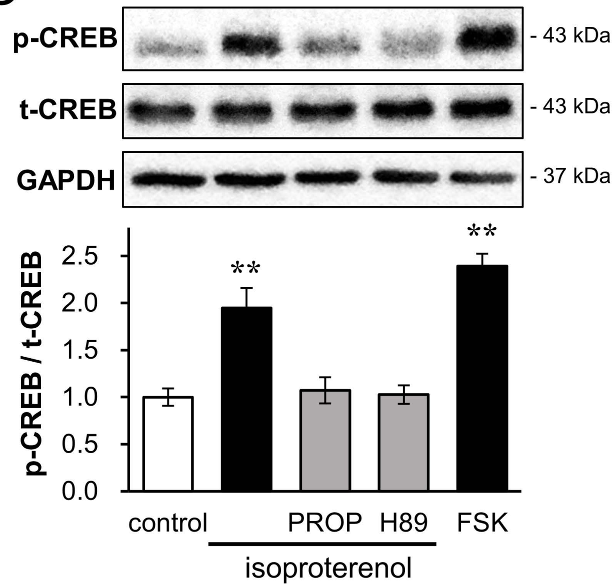
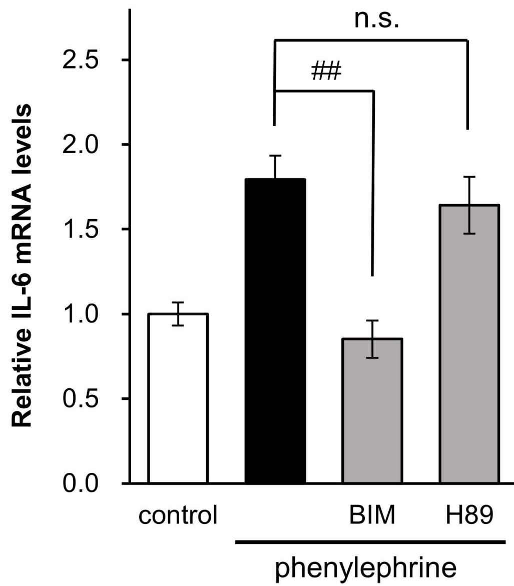
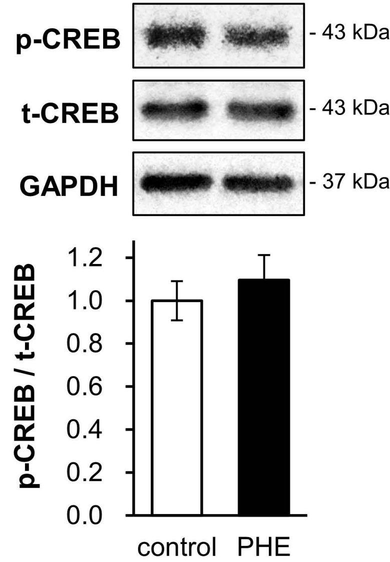
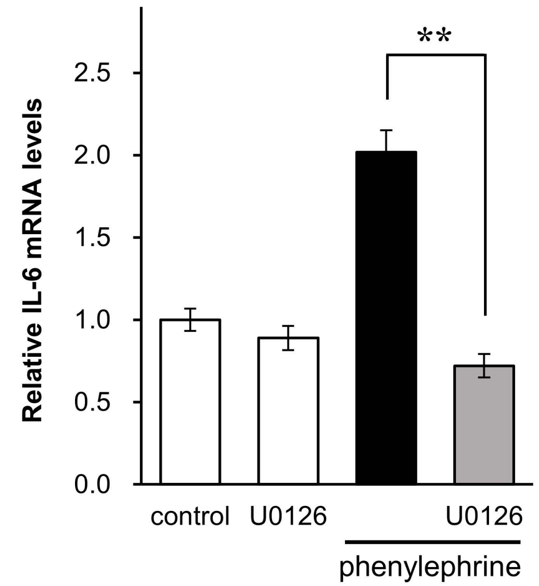
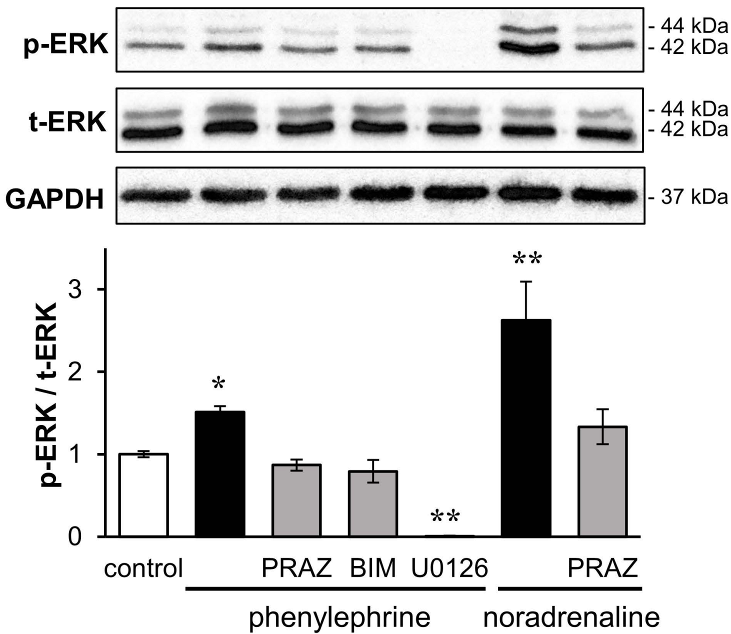


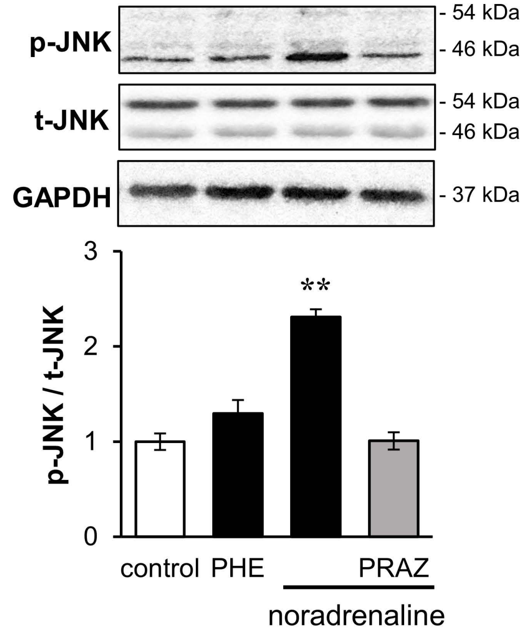
Fig. 3











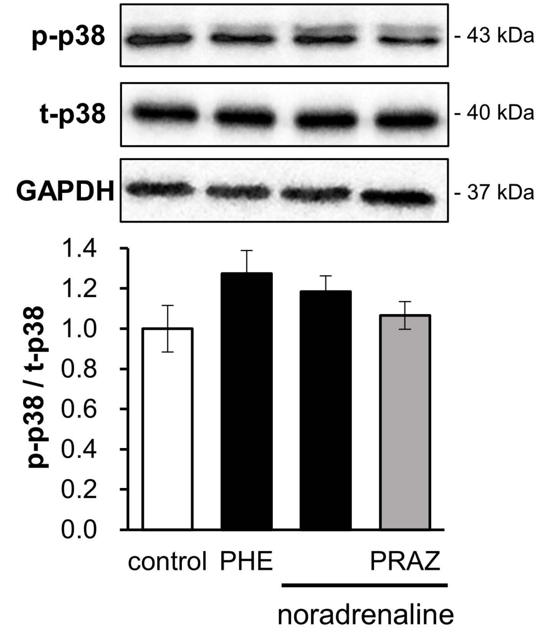


Fig. 4

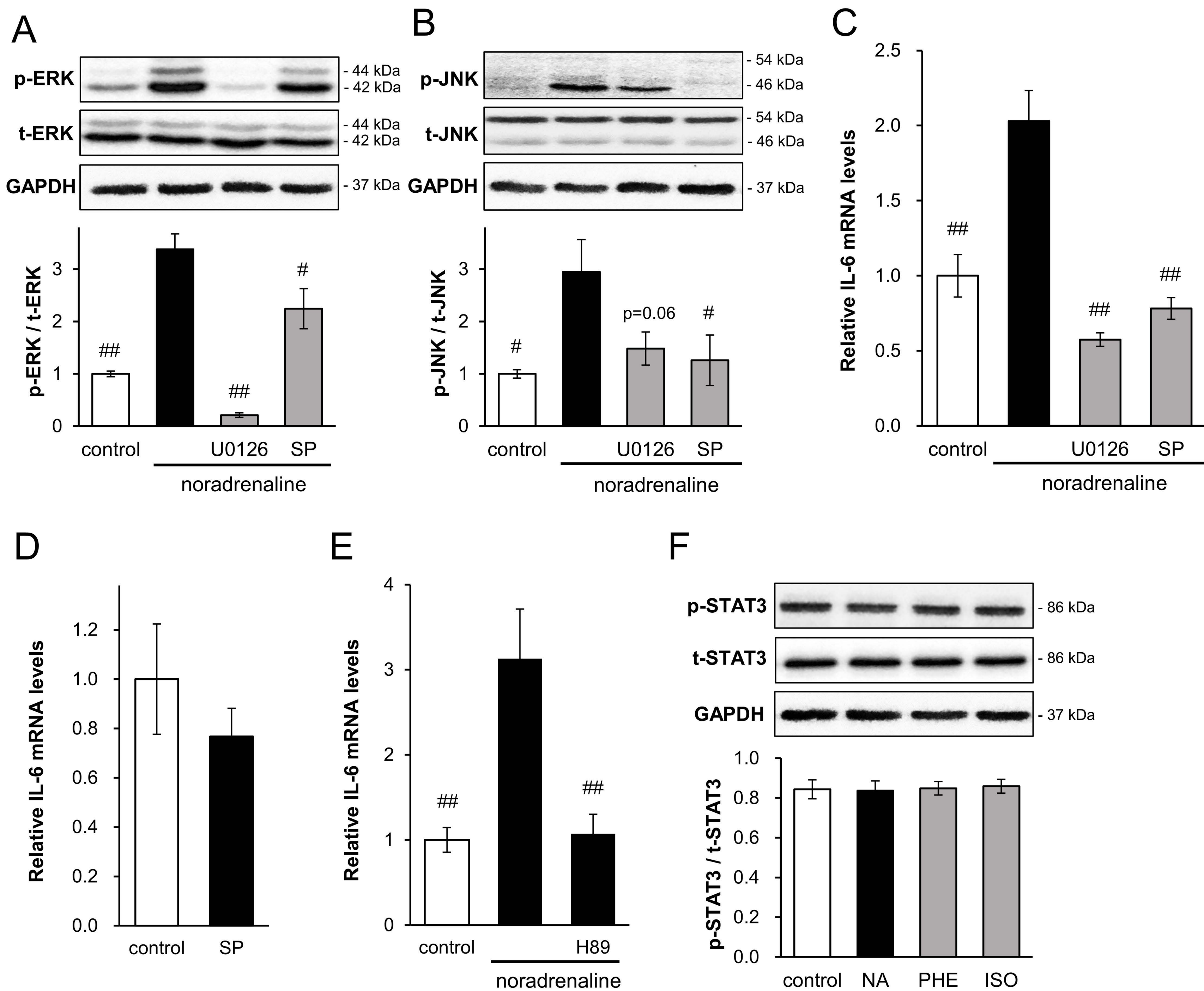


Fig. 5