ELSEVIER

European Journal of Pharmacology 285 (1995) 61–67

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Enhancement of 5-hydroxytryptamine-stimulated phosphoinositide hydrolysis in the rat cerebral cortex by repeated immobilization stress

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Received 17 May 1995; revised 16 June 1995; accepted 20 June 1995

Abstract

The present study was undertaken to investigate the influence of repeated immobilization stress on phosphoinositide hydrolysis induced by 5-hydroxytryptamine (5-HT) and noradrenaline in the rat cerebral cortex. Three groups of rats subjected to stress intervention were immobilized for 2 h per day for 3, 7 and 14 days. The stress intervention of any duration did not affect noradrenaline-stimulated phosphoinositide hydrolysis. The 3- and 7-day repeated immobilization enhanced 5-HT-stimulated phosphoinositide hydrolysis, whereas the characteristics of 5-HT_2 receptor binding did not change. Chronic treatment with imipramine partially, but significantly, suppressed the increase in 5-HT-stimulated phosphoinositide hydrolysis, induced by the 3-day repeated immobilization. These findings imply that modulation of 5-HT-stimulated phosphoinositide hydrolysis occurs in stressful situations and that the therapeutic effects of tricyclic antidepressant drugs might be related to the modulation of phosphoinositide hydrolysis mediated by 5-HT receptors.

Keywords: Phosphoinositide hydrolysis; Immobilization stress; 5-HT (5-hydroxytryptamine, serotonin); Imipramine

1. Introduction

It is well known that antidepressant drugs enhance the availability of serotonin (5-hydroxytryptamine, 5-HT) and/or noradrenaline in the synaptic cleft by inhibiting the uptake of these neurotransmitters and subsequently inducing down-regulation of receptors. The therapeutic action of antidepressants is supposed to be attributable to these pharmacological properties. Several newly developed antidepressants show an inhibitory action on phosphoinositide hydrolysis, which is involved with an intracellular second messenger system, without causing any changes in membrane receptors (Sanders-Bush et al., 1989). In addition, lithium, which is used for the treatment of affective disorders, has recently been reported to alter the receptor-mediated regulation of adenylate cyclase activity and phosphoinositide hydrolysis in the brain. Namely, lithium suppresses phosphoinositide hydrolysis by inhibiting inositol-1-phosphatase activity (Berridge et al., 1983), and it also inhibits the isoproterenol- and carbachol-induced increase in GTP-binding in the rat cerebral cortex (Avissar et al., 1989). These findings suggest that a dysfunction of membrane receptors and/or of the signal transduction process including phosphoinositide hydrolysis may play an important role in the pathogenesis of affective disorders. We have studied the alteration in phosphoinositide hydrolysis caused by acute stress and showed that the single forced swimming test changed 5-HT-stimulated phosphoinositide hydrolysis (Kawanami et al., 1992). However, a single immobilization stress did not alter noradrenaline- and carbachol-stimulated phosphoinositide hydrolysis in the rat cerebral cortex (Morinobu et al., 1992). In this context, it is considered that the influence of repeated stress on phosphoinositide hydrolysis may be more relevant with respect to the clinical setting. The present study was, therefore, undertaken to investigate the influence of repeated immobilization stress on noradrenaline- and 5-HT-stimulated phosphoinositide hydrolysis and the influence of chronic treatment with imipramine on the stress-induced changes in phosphoinositide hydrolysis in the rat cerebral cortex.

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SSDI 0014-2999(95)00381-9

2. Materials and methods

2.1. Drugs

Imipramine was kindly provided by Ciba-Geigy Japan (Hyogo, Japan). 5-HT sulphate, mianserin hydrochloride, noradrenaline hydrochloride, pargyline hydrochloride, prazosin hydrochloride and serum bovine albumin were purchased from Sigma Chemical Co. (St. Louis, USA). *myo*-[^{3}H]Inositol (18.3 Ci/mmol) was purchased from Amersham Radiolabeled Chemicals (Buckinghamshire, UK). [^{3}H]Ketanserin (77.8 Ci/mmol) was purchased from New England Nuclear (Boston, USA) and [^{3}H]prazosin (84.0 Ci/mmol) from Amersham Japan (Tokyo, Japan). All other chemicals were purchased from Wako Chemicals Co. (Tokyo, Japan).

2.2. Animals and treatment

Male Wistar rats (weighing ca. 200 g before the beginning of the 2-week prestress period) were purchased from Charles River Company. Rats were housed four or five per cage with food and water available ad libitum. For chronic pretreatment with an antidepressant drug, rats were injected intraperitoneally (i.p.) with imipramine (10 mg/kg), dissolved in saline, once daily for 14 days before and during the stress intervention. During stress, imipramine was administered to rats immediately before immobilization. Rats in the control group were injected with saline.

2.3. Repeated immobilization stress test

For the stress intervention, acrylic restrainers (7 cm in diameter, 15 cm in length) were used. Three groups of rats were subjected to the stress intervention and they were immobilized for 2 h per day (09.00–11.00 h) for 3, 7 and 14 days. Control rats were left in their home cages.

2.4. Changes in body weight

Rats were weighed every day prior to the stress intervention, and the differences in weight are presented as the gain in weight from the pre-stress weight.

2.5. Preparation of cerebral cortex slices and determination of [^{3}H]inositol 1-phosphate (IP_1) and [^{3}H]inositol phosphates

Rats were decapitated immediately after the last immobilization, their brains were rapidly removed and cross-chopped slices (ca. 350 μm) were prepared from the cerebral frontal cortex according to the procedure described previously (Kawanami et al., 1992). The slices were preincubated for 60 min in a modified Krebs-Henseleit (KH) buffer (in mM: 118 NaCl; 4.7 KCl; 2.55 $CaCl_2$; 1.18 KH_2PO_4; 1.18 $MgSO_4$; 24.88 $NaHCO_3$; 11.1 glucose), equilibrated with 95% O_2-5% CO_2 at 37°C (pH 7.4).

[^{3}H]Inositol phosphates were determinated according to the methods of Berridge et al. (1983) and Brown et al. (1984) with minor modifications (Kawanami et al., 1992). The slices were washed extensively with KH buffer and then they were incubated in 250 μl of KH buffer that contained 2 μCi *myo*-[^{3}H]inositol per slice at 37°C for 90 min under the same oxygenation conditions. Radiolabeled slices were washed and incubated with agonists in a final volume of 270 μl of KH buffer that contained 7.5 mM LiCl at 37°C for 45 min under the same oxygenation conditions. The reaction was terminated by addition of 1 ml of 5% trichloroacetic acid. The reaction mixtures were centrifuged at 2400 rpm for 10 min and 750 μl of the supernatant was taken for the assay of [^{3}H]inositol phosphates. The slices were homogenized for 1 min in a Polytron PT-10/35 (Kinematica, Luzern, Switzerland) after the addition of 1 ml of trichloroacetic acid and centrifuged at 2100 rpm for 2 min. An aliquot of 750 μl of the supernatant was removed and added to the previously collected 750 μl. Trichloroacetic acid was removed from the solution by washing 3 times with three volumes of water-saturated diethylether. The pellets were used for the measurement of tissue protein by the method of Lowry et al. (1951) and for the estimation of *myo*-[^{3}H]inositol incorporation into phospholipids.

Samples were added to columns that contained 750 μl of Dowex-1 resin (AG-1X8 100–200 mesh, formate form) and phosphate esters were eluted by stepwise addition of formate solutions of increasing strength. The columns were washed with 10 ml of distilled water to remove free inositol. Then glycerophosphoinositol and inositol 1:2 cyclic phosphate were eluted with 5 mM sodium tetraborate/60 mM sodium formate. Inositol 1-phosphate (IP_1) was eluted with 200 mM ammonium formate/100 mM formate, inositol 1,4-bisphosphate (IP_2) with 450 mM ammonium formate/100 mM formate, and inositol 1,4,5-tris-phosphate (IP_3) with 1 M ammonium formate/100 mM formate. Samples (8 ml) of the extracts were added to 8 ml of scintillator ACS-II (Amersham Radiolabeled Chemicals) and their radioactivity was counted. Inositol phosphates represents all inositol phosphates assayed separately and then calculated from the results ($IP_1 + IP_2 + IP_3$). All data were normalized to *myo*-[^{3}H]inositol incorporated and are expressed as percentages relative to the basal [^{3}H]inositol phosphate level in the absence of agonists.

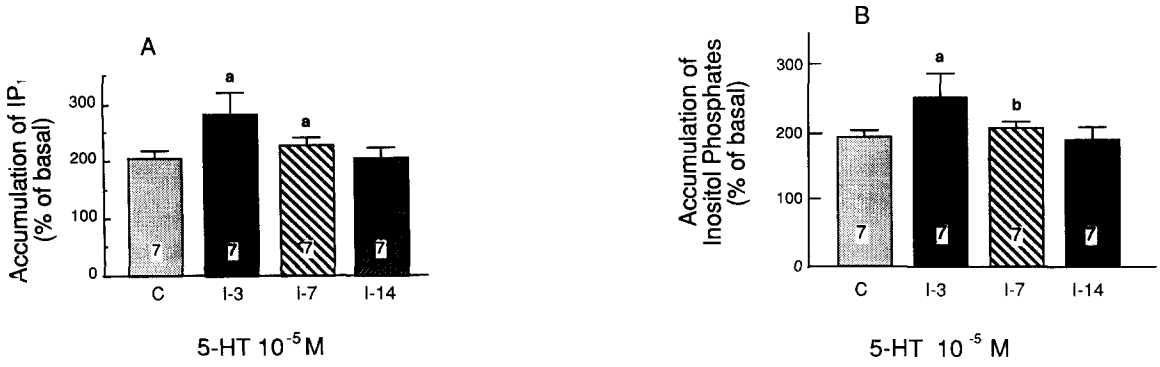

Fig. 1. Influence of repeated immobilization stress for various durations on 5-HT-stimulated [^{3}H]IP$_1$ (A) and [^{3}H]inositol phosphates (B) accumulation in rat cerebral cortex slices. Data are expressed as a percentage over the basal level in the absence of 5-HT and presented as means ± S.E.M. (number of experiments in columns). The basal radioactivities of [^{3}H]IP$_1$ accumulation were 8000 ± 1956 dpm/mg protein for the control, 8402 ± 1654 dpm/mg protein for I-3, 8480 ± 1675 dpm/mg protein for I-7 and 7946 ± 1834 dpm/mg protein for I-14. The basal radioactivities of [^{3}H]inositol phoaphates accumulation were 9848 ± 2265 dpm/mg protein for the control, 10629 ± 1786 dpm/mg protein for I-3, 10305 ± 1928 dpm/mg protein for I-7 and 9842 ± 2264 dpm/mg protein for I-14. $^aP < 0.01$, $^bP < 0.05$ vs. the control accumulation; vertical bars: S.E.M.

2.6. Receptor binding assay

Rats were decapitated immediately after the last immobilization; the frontal cortex was removed and stored at −80°C until assayed. The 5-HT$_2$ receptor binding assay was performed according to the method of Leysen et al. (1982) with a partial modification. In brief, the frontal cortex was weighed and homogenized in 50 volumes of Tris-HCl buffer (50 mM, pH 7.6) in a Polytron PT-10/35 at setting 7 for 15 s. The homogenate was centrifuged at 50000 × g for 10 min at 4°C. The supernatant was discarded; the pellet was suspended in the same buffer, and the suspension was centrifuged again. The final pellet was suspended in 50 volumes of Tris-HCl buffer and used as the crude membrane fraction for the receptor binding assay. The reaction mixture for the 5-HT$_2$ receptor binding assay consisted of 0.1 ml of the membrane fraction, 0.1 ml of [^{3}H]ketanserin solution, 0.7 ml of Tris-HCl buffer (pH 7.6) and 0.1 ml of buffer or mianserin (1 μM). The final concentration of [^{3}H]ketanserin for saturation assays was 0.2–5 nM. After incubation for 30 min at 25°C, the reaction mixture was rapidly filtered under vacuum through a Whatman glass filter (GF/C) and the filter was washed 3 times with ice-cold Tris-HCl buffer (pH 7.4). The filter was transferred to a counting vial that contained 4 ml of scintillation mixture ACS-II.

The α_1-adrenoceptor binding assay was performed according to the method of Greengrass and Bremner (1979) with a partial modification. The tissue was prepared by the same procedure as for the 5-HT$_2$ receptor binding assay. The assay mixture consisted of 0.1 ml of membrane fraction, 0.1 ml of [^{3}H]prazosin solution, 0.7 ml of Tris-HCl buffer (pH 7.6) and 0.1 ml of buffer or a solution of prazosin (1 μM). The final concentration of [^{3}H]prazosin for saturation assays was 0.05–5 nM.

Radioactivity was measured by liquid scintillation spectroscopy in 8 ml of ACS-II. Specific binding was defined as the difference between the amount of radioactive ligand that bound in the presence and the amount that bound in the absence of mianserin (1 μM) or prazosin (1 μM), respectively. The dissociation constant (K_d) and the maximum binding capacity (B_{max}) were calculated from linear regression analysis of Scatchard plots. Tissue protein content was measured according to the method of Lowry et al. (1951).

2.7. Data analysis

The significance of differences between groups was evaluated by one-way analysis of variance (ANOVA) and the significance of differences between two groups was evaluated by Student's t-test for unpaired values. Differences between mean values were considered to be significant when the P value was smaller than 0.05.

3. Results

3.1. Changes in body weight

Changes in body weight during repeated exposure to immobilization stress are shown in Table 1. Compared

Table 1
Influence of repeated immobilization for various durations on gain in weight (g) of rats

	Weight gain (g)		
Days exposed to IMS	3 days	7 days	14 days
Control	15.6 ± 1.4	39.7 ± 1.5	77.8 ± 2.0
IMS	−4.5 ± 3.4 [a]	6.4 ± 1.3 [a]	24.6 ± 1.1 [a]

Data are presented as means ± S.E.M. [a] $P < 0.01$ vs. the control group ($n = 7$ for each group). IMS: immobilization stress.

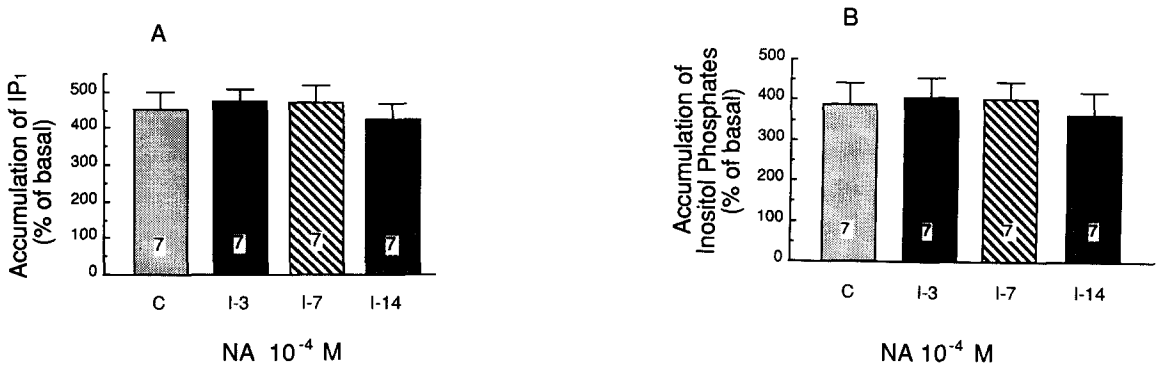

Fig. 2. Influence of repeated immobilization stress for various durations on noradrenaline-stimulated [^{3}H]IP_1 (A) and [^{3}H]inositol phosphates (B) accumulation in rat cerebral cortex slices. Data are expressed as a percentage over the basal level in the absence of noradrenaline and presented as means ± S.E.M. (number of experiments in columns). The basal radioactivities of [^{3}H]IP_1 accumulation were 8000 ± 1956 dpm/mg protein for the control, 8402 ± 1654 dpm/mg protein for I-3, 8480 ± 1675 dpm/mg protein for I-7 and 7946 ± 1834 dpm/mg protein for I-14. The basal radioactivities of [^{3}H]inositol phosphates accumulation were 9848 ± 2265 dpm/mg protein for the control, 10629 ± 1786 dpm/mg protein for I-3, 10305 ± 1928 dpm/mg protein for I-7 and 9842 ± 2264 dpm/mg protein for I-14. Vertical bars: S.E.M.

with the control group, the stress group showed a loss of body weight on day 3. Subsequently, however, the stress group began to gain weight, reaching the pre-stress level on day 4 (data not shown). After the initial drop in body weight, its later increase was significantly lower than in the controls and the weight remained below the control level during the stress intervention.

3.2. Effects of repeated immobilization stress on the [^{3}H]IP_1 and [^{3}H]inositol phosphates accumulation stimulated by 5-HT and noradrenaline

In the rat cerebral cortex slices prelabeled with *myo*-[^{3}H]inositol, both 5-HT and noradrenaline increased [^{3}H]IP_1 and [^{3}H]inositol phosphates accumulation in a concentration-dependent manner (Kuwayama et al., 1991; Kawanami et al., 1992; Morinobu et al., 1992). The [^{3}H]IP_1 accumulation induced by 5-HT and noradrenaline reached a maximal level at 10^{-5} M and 10^{-4} M with EC_{50} values of 4.7×10^{-7} M and 1.4×10^{-5} M, respectively. The concentration-response relationships for [^{3}H]inositol phosphates accumulation induced by 5-HT and noradrenaline were very similar to those for [^{3}H]IP_1. The influence of long-lasting immobilization stress on the phosphoinositide hydrolysis acceleration in response to 10^{-5} M 5-HT and 10^{-4} M noradrenaline was investigated in subsequent experiments. A single exposure to immobilization stress for 2 h did not cause any significant changes in the [^{3}H]IP_1 and [^{3}H]inositol phosphates accumulation induced by 5-HT and noradrenaline (data not shown).

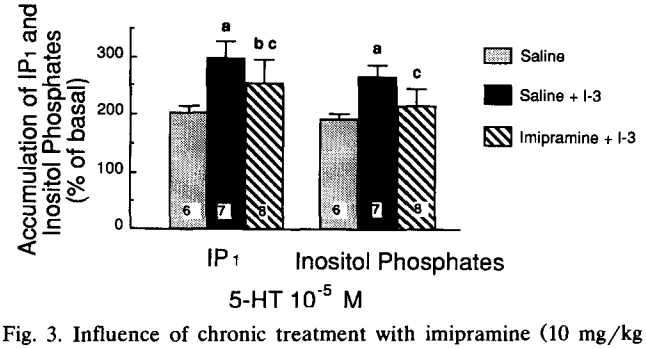

Fig. 3. Influence of chronic treatment with imipramine (10 mg/kg i.p. per day) on the increase in 5-HT-stimulated phosphoinositide hydrolysis induced by the 3-day repeated immobilization stress. Data are expressed as a percentage over the basal [^{3}H]IP_1 and [^{3}H]inositol phosphates levels in the absence of 5-HT and presented as means ± S.E.M. (number of experiments in columns). The basal radioactivities of [^{3}H]IP_1 accumulation were 8286 ± 1846 dpm/mg protein for saline, 8547 ± 1618 dpm/mg protein for saline + I-3 and 8455 ± 1350 dpm/mg protein for imipramine + I-3. The basal radioactivities of [^{3}H]inositol phosphates accumulation were 10173 ± 2147 dpm/mg protein for saline, 10611 ± 1844 dpm/mg protein for saline + I-3 and 10607 ± 1579 dpm/mg protein for imipramine + I-3. $^aP < 0.01$, $^bP < 0.05$ vs. saline; $^cP < 0.05$ vs. saline + I-3; vertical bars: S.E.M.

Rats exposed to repeated immobilization stress were divided into three groups. Each group was subjected to stress for 2 h per day for 3, 7 and 14 consecutive days and designated I-3, I-7 and I-14, respectively. Fig. 1A shows the effects of repeated immobilization stress for various durations on 5-HT-stimulated [^{3}H]IP_1 accumulation. [^{3}H]IP_1 accumulation in the control group was 205.6% ($n = 7$). A significant increase in [^{3}H]IP_1 accumulation in response to 10^{-5} M 5-HT was observed in groups I-3 and I-7. There was, however, no significant increase in [^{3}H]IP_1 accumulation in group I-14. Identical results were obtained for [^{3}H]inositol phosphates accumulation in response to 5-HT (Fig. 1B). [^{3}H]Inositol phosphates accumulation in the control group was 193.3% ($n = 7$). A significant increase in the 5-HT-induced [^{3}H]inositol phosphates accumulation was found in groups I-3 and I-7, while no significant increase was found in group I-14.

Fig. 2 shows the effects of repeated immobilization for various durations on noradrenaline-stimulated [^{3}H]IP_1 and [^{3}H]inositol phosphates accumulation.

Table 2
Effects of repeated immobilization for various durations on [^{3}H]ketanserin binding to the membrane fraction derived from rat cerebral cortex

	n	B_{max} (fmol/mg protein)	K_d (nM)
Control	6	116.65 ± 9.67	0.53 ± 0.09
I-3	5	117.03 ± 7.04	0.69 ± 0.09
I-7	5	114.71 ± 5.26	0.55 ± 0.12
I-14	6	119.19 ± 7.34	0.57 ± 0.07

Data are presented as means ± S.E.M. determined in duplicate.

[^{3}H]IP_1 accumulation in the control group was 452.9% ($n = 7$). There was no significant change in noradrenaline-stimulated [^{3}H]IP_1 accumulation in groups I-3, I-7 and I-14 (Fig. 2A). While the [^{3}H]IP_1 accumulation in group I-14 showed a tendency to decrease, the change was not statistically significant. Substantially identical results were obtained for noradrenaline-stimulated [^{3}H]inositol phosphates accumulation in the stressed groups (Fig. 2B). [^{3}H]Inositol phosphates accumulation in the control group was 386.9% ($n = 7$). No significant changes were observed in groups I-3, I-7 and I-14.

3.3. Influence of repeated immobilization stress on [^{3}H]ketanserin and [^{3}H]prazosin binding

The influence of repeated immobilization stress on 5-HT_2 receptors and α_1-adrenoceptors was studied with [^{3}H]ketanserin (Table 2) and [^{3}H]prazosin (Table 3), respectively. No significant differences in B_{max} and K_d values were found between the control group and the stress group. These results indicate that neither the density nor the affinity of 5-HT_2 receptors as well as of α_1-adrenoceptors changed in the rat cerebral cortex during repeated immobilization stress for up to 14 days.

3.4. Influence of chronic treatment with imipramine on the change in phosphoinositide hydrolysis induced by the 3-day repeated immobilization stress

Since 5-HT-stimulated phosphoinositide hydrolysis was enhanced most prominently in group I-3, we examined whether chronic treatment with an antidepressant drug, namely imipramine, modulated the 5-HT-induced change. Rats were treated with imipramine for 14 days and then were exposed to the 3-day repeated immobilization. Treatment with imipramine was continued during the days when stress was applied. As shown in Fig. 3, 5-HT-stimulated [^{3}H]IP_1 accumulation in rats treated with imipramine + stress (252.9%, $n = 8$) was significantly lower than that in rats treated with saline + stress (296.8%, $n = 7$). The [^{3}H]IP_1 accumulation in the imipramine-treated group was still significantly higher than that in rats treated only with saline. Substantially identical results were obtained for [^{3}H]inositol phosphates accumulation: 213.8% in the imipramine + stress group; 265.2% in the saline + stress group. These results indicate that the treatment with imipramine partially reversed the enhancement of 5-HT-stimulated phosphoinositide hydrolysis induced by the 3-day repeated immobilization.

Table 3
Effects of repeated immobilization for various durations on [^{3}H]prazosin binding to the membrane fraction derived from rat cerebral cortex

	n	B_{max} (fmol/mg protein)	K_d (nM)
Control	5	47.54 ± 1.50	0.31 ± 0.03
I-3	5	53.29 ± 2.78	0.39 ± 0.05
I-7	5	58.62 ± 3.98	0.41 ± 0.04
I-14	5	54.62 ± 1.18	0.36 ± 0.01

Data are presented as means ± S.E.M. determined in duplicate.

4. Discussion

A remarkable loss of body weight was observed on day 3 of the repeated immobilization stress, and the rate of increase in body weight in the stress group was lower than that in the control group even on day 7. These observations suggest that rats were kept in a stressful condition during the repeated immobilization, even though the response to stress diminished, probably due to adaptation mechanisms. These results are in agreement with those of previous behavioral studies, in which stress adaptation occurred during repeated immobilization (Kennet et al., 1985). Since an alteration in phosphoinositide hydrolysis induced by a chronic stressful situation has not been addressed previously, this is the first report showing the differential influence of chronic stress on 5-HT- and noradrenaline-stimulated phosphoinositide hydrolysis in rats subjected to repeated immobilization.

5-HT-stimulated phosphoinositide hydrolysis was enhanced most prominently by the 3-day repeated immobilization, when the weight loss was most prominent. 5-HT-stimulated response was significantly increased by the 7-day, but not by the 14-day repeated immobilization. Since the 5-HT_2 receptor binding characteristics were not altered by the repeated immobilization, it is postulated that the increase in 5-HT-stimulated phosphoinositide hydrolysis might have stemmed from an enhancement of the intracellular signal transduction system subsequent to receptor activation. However, it is reported that the 5-HT_{2C} receptor is linked to phosphoinositide hydrolysis, although its population is smaller than that of the 5-HT_{2A} receptor in the frontal cortex. In this context, the influence of repeated immobilization stress on the 5-HT_{2C} receptor binding characteristics should be considered. In a

behavioral study, Kennet et al. (1985) reported that when rats were immobilized repeatedly for 7 days, the occurrence of body shakes increased, a phenomenon that was postulated to be due to 5-HT_2 receptor-dependent behavior, although the exact mechanism remains unknown at present. In the present study, the repeated immobilization induced no alteration in α_1-adrenoceptor-mediated phosphoinositisde hydrolysis, an indication that α_1-mediated phosphoinositide hydrolysis is not involved in the modulation caused by the repeated immobilization stress in the rat cerebral cortex. By contrast, it has been reported that repeated immobilization stress decreased β- and α_2-adrenoceptor density in the rat cerebral cortex (Stone and Platt, 1982; Lynch et al., 1983). The alterations in the receptor-mediated cyclic AMP accumulation induced by chronic stress might have modulated 5-HT-stimulated phosphoinositide hydrolysis via a putative intracellular cross-talk mechanism.

Treatment with imipramine significantly suppressed the increase in 5-HT-stimulated phosphoinositide hydrolysis after the 3-day repeated immobilization, while the response to 5-HT was still greater than that in the control rats without the 3-day repeated immobilization. These results indicate that the treatment with imipramine partially inhibited the enhancement of 5-HT-stimulated phosphoinositide hydrolysis induced by the repeated immobilization stress. In previous studies, the effects of chronic treatment with an antidepressant drug on 5-HT-mediated signal transduction were controversial. Kendall and Nahorski (1985) reported that chronic, but not acute, treatment of rats with imipramine (10 mg/kg) resulted in a reduction in 5-HT-induced [^{3}H]IP_1 accumulation in the cerebral cortex, which is in line with the present findings. Goodwin et al. (1986) showed that chronic treatment with lithium reduced the number of head twitches mediated by 5-HT_2 receptors in mice, while Kusumi et al. (1991) failed to detect any significant modulation of the 5-HT-induced response by imipramine in the rat hippocampal slices. The down-regulation of 5-HT_2 receptors induced by chronic treatment with imipramine might prevent the significant increase in 5-HT-stimulated phosphoinositide hydrolysis induced by the 3-day repeated immobilization stress.

Antidepressant drugs act not only on the reuptake of monoamines but also on other processes of intracellular signal transduction. Avissar et al. (1989) demonstrated an inhibitory effect of lithium on [^{3}H]GTP binding, while Ozawa and Rasenick (1989) reported that chronic treatment with the antidepressant drug enhanced receptor-effector coupling via an effect on G protein. It is tempting, therefore, to speculate that the chronic treatment with imipramine might similarly influence points distal to the receptor to some extent, in addition to the characteristics of receptor binding, in the present experimental system. Our findings imply that the stress-induced enhancement of 5-HT-stimulated phosphoinositide hydrolysis also involves an imipramine-resistant process.

In summary, repeated immobilization stress for 3 and 7 days induced an increase in 5-HT-stimulated phosphoinositide hydrolysis in the rat cerebral cortex slices. Noradrenaline-stimulated phosphoinositide hydrolysis was unaffected by the repeated immobilization stress. Chronic treatment with imipramine significantly diminished the extent of the change induced by the stress intervention. The present findings support the hypothesis that modulation of 5-HT-stimulated phosphoinositide hydrolysis might be involved in the mechanism of affective disorders and is also responsible for the mechanism of action of imipramine.

Acknowledgements

The authors thank Professor K. Kato, M.D., Ph.D., Department of Physiology, Yamagata University School of Medicine, for his continuous encouragement and N. Kuwayama, M.D., Ph.D., Department of Neuro-Psychiatry, Yamagata University School of Medicine, for his technical advice throughout this study.

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