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Effects of Paroxetine on the Desensitization of 5-HT_{1A} and 5-HT_{1B} Receptors

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= 국문초록 =

5-HT_{1A}와 5-HT_{1B} 수용체의 탈감작에 대한 Paroxetine의 효과

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연구목적 : 5-HT_{1A}와 5-HT_{1B} 수용체의 탈감작은 paroxetine이나 다른 세로토닌 재흡수 억제제를 장기간 투여하였을 때 치료 효과를 유도하는 기전으로 알려져 있다. 본 연구에서는 세로토닌 재흡수 억제제인 paroxetine과 5-HT_{1A} 수용체 길항제인 *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY-100635)을 함께 반복 투여하였을 때 전두엽 피질에서 세로토닌 농도와 5-HT_{1A}와 5-HT_{1A} 수용체의 활성을 조사하여 5-HT_{1A} and 5-HT_{1B} 수용체의 탈감작을 조절하는 기전이 동일한지를 연구하였다.

연구방법 : 수컷 Wistar 쥐에 paroxetine 5mg/kg를 12일간 매일 복강 주사하고, Way-100635 0.3 mg/kg는 paroxetine 주사 1시간 후에 피하 주사하였다. microdialysis 실험은 마지막 주사 1일 후에 시행하였다. 5-HT_{1A} 수용체의 탈감작 효과를 측정하기 위해서는 5-HT_{1A} 수용체 agonist인 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT)을, 그리고 5-HT_{1B} 수용체의 탈감작 효과를 측정하기 위해서는 5-HT_{1B} 수용체 agonist인 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-*b*]pyrid-5-one (CP 93129)를 사용하였다. 세로토닌 농도는 BAS-HPLC로 측정하였다.

연구결과 : Paroxetine이 전두엽 피질에서 5-HT_{1A}수용체에 대한 탈감작과 5-HT_{1B} 수용체에 대한 탈감작 효과는 5-HT_{1A} 수용체 길항제인 WAY-100635에 의해 억제되었다.

결 론 : 전두엽 피질에서 세포외 세로토닌 농도의 조절은 서로 다른 자가조절 기전에 의한다.

중심 단어 : 세로토닌(5-hydroxytryptamine, 5-HT) · Paroxetine · 5-HT_{1A} 수용체 · 5-HT_{1B} 수용체.

Introduction

Selective serotonin (5-HT) reuptake inhibitors have become very popular because of their effectiveness in treating several mood disorders, including depression, anxiety, and eating disorders. However, the delay after onset of administration until a therapeutic effect is observed remains one of the major problems associated with the use of antidepressant drugs in the treatment of depression. A further problem is that many patients do not respond to any of the drugs in clinical use. Several

strategies have been proposed to overcome these problems, notably the use of potentiating agents, which themselves may not have therapeutic effects, to augment or accelerate the effects of established antidepressants. The observation made in many laboratories-that the effect of acute administration of a selective serotonin reuptake inhibitor such as paroxetine to increase synaptic 5-HT levels is potentiated by simultaneous administration of an antagonist of 5-HT_{1A} or 5-HT_{1B} autoreceptors-resulted in the development of pindolol as an augmenting or accelerating agent. It was reasoned[1] that antagonist administration mimicked the effect of chronic administration of a selective serotonin reuptake inhibitor, which induces desensitization of both types of autoreceptor[2-4]. However, clinical trials with pindolol were equivocal (for reviews, see)[18][20]. In addition, the mechanism proposed for the action of this agent[1] was based on the results of acute animal experiments only, and did not take into account the fact that pindolol and the selective serotonin reuptake inhibitor must be taken repeatedly before a therapeutic action is observed. More recently, three studies have investigated the effects of chronic (2 week) administration of paroxetine in combination with either pindolol, which is in fact a partial agonist at presynaptic 5-HT_{1A} autoreceptors and an antagonist at 5-HT_{1B} autoreceptors, or the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY-100635) (Dawson et al., 2000). observed that while either pindolol or paroxetine given alone desensitized 5-HT_{1A} autoreceptors, in the presence of paroxetine, pindolol prevented the paroxetine-induced desensitization. Similar results were obtained by[5] using WAY-100635.

In the present work, I have determined the effects of 12-day combined paroxetine and WAY-100635 administration on basal 5-HT levels and 5-HT_{1A} and 5-HT_{1B} autoreceptor activity in the frontal cortex and hypothalamus. Although it is now established that activation of postsynaptic 5-HT_{1A} receptors on cortical-probably glutamatergic-neurons may also inhibit firing of serotonergic neurons and thus exert a negative effect on 5-HT release in nerve terminal areas[6][7], the term "5-HT_{1A} autoreceptors" will be used in this paper to denote both the 5-HT_{1A} autoreceptors located somatodendritically on serotonergic neurons in the raphe nuclei and the subset of postsynaptic 5-HT_{1A} receptors in the cortex that influences 5-HT release.

Materials and Methods

1. Treatment of animals

Male Wistar rats were used in all experiments. The rats were housed by treatment group in a temperature-controlled environment (24℃) with a regular 12h light/dark cycle. Food and water were freely available. Paroxetine was administered at 5mg/kg by intraperitoneal injection on each treatment day, and WAY-100635 at 0.3mg/kg was administered 1h later by subcutaneous injection. This procedure was adopted instead of simultaneous injections since[8] showed in acute experiments that the increase in cortical 5-HT levels obtained when WAY-100635 was injected 80min after paroxetine was more than twice that observed when the two compounds were coadministered. Injections were begun in a staggered manner so that a 3-day experimental period (1 day for implantation of guides and probes and 2 days for collection of fractions) was available for each pair of animals.

2. Implantation and perfusion of microdialysis probes

The microdialysis experiments were performed simultaneously on three animals, using a Bioanalytical Systems (BAS) "Raturn" interactive awake animal system. One day after the last injection, animals were anaesthesised with a 17 : 3 mixture of ketamine (100mg/ml) and xylazine (2%) and mounted in a stereotaxic apparatus. Guides for dialysis probes (CMA/12) were implanted into the frontal cortex at anterior 3.2mm from bregma, 2.5mm lateral, and 2.0mm vertical, and into anterior hypothalamus at anterior 1.5mm from bregma, 1.3mm lateral, and 7.0mm vertical. A subcutaneous cannula was also implanted at the back of the neck and secured to the skull with screws and dental acrylic. The rats were maintained under anesthesia for approximately 1 h, after which they were free-moving and had unlimited access to food and water. Dialysis probes (4mm for cortex and

2mm for hypothalamus) were inserted into the guides towards the end of the period of anesthesia. The inlets of the probe were connected, through plastic tubing with an internal volume of 12 μl/m, to 1-ml gas-tight syringes mounted on a microinfusion pump. The inlet and outlet tubing of the probe were mounted to a steel wire running from the head of the rat to a balanced arm. Movement of the turntable of the "Raturn" allowed the animal to rotate and rear without entangling the fluid tubing. The probes were perfused with Ringer's solution containing 2.25 mM $CaCl_2$, 4mM KCl, 147mM NaCl, and 10 μM citalopram, pH 6.5, at 0.2 μl/min overnight. The following morning, the flow rate was increased to 0.5 μl/min, and 30-min fractions were collected. After each experiment, the dialysis probes were removed under anesthesia, sterilised in alcohol, and, if still intact, reinserted into new animals. The animal procedures outlined above received the approval of the Institutional Animal Care and Use Committee of the Hebrew University Faculty of Medicine and Dental Medicine and Hadassah Medical Organization.

3. 5-HT receptor challenges

On the second experimental day for each animal, fractions were injected into the high-performance liquid chromatography (HPLC) apparatus immediately after collection for measurement of 5-HT. Once stable baseline 5-HT levels had been obtained, usually after collecting four or five experimental samples, the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT ; 50 μg/kg), was injected via the subcutaneous cannula. A further six fractions were then collected. On the following day, once stable baseline 5-HT levels had been obtained, the 5-$HT_{1B/1D}$ receptor agonist, 3-(1,2,5,6-tetrahydropyrid-4-yl) pyrrolo [3,2-*b*] pyrid-5-one (CP 93129), at a concentration of 10 μM was infused into the cortex and into the hypothalamus via the microdialysis probes during two fractions (i.e., for 60min) and a further four or five fractions were collected.

4. Determination of 5-HT levels

Concentrations of 5-HT were determined by a BAS HPLC system. Samples were injected immediately after collection using a Rheodyne 9125 injector with a 5-μl injection loop. The mobile phase was made up of 90mM sodium dihydrogen phosphate, 10mM NaCl, 0.5mM EDTA, 0.15g/l sodium octyl sulphate, and 10.5% acetonitrile, pH 5, and was delivered by the HPLC pump at 1.0ml/min. The mobile phase was passed through a flow splitter and pumped through a 10cm C-18 5-mm reversed-phase column at 0.1ml/min. 5-HT content was analysed with a LC-4C electrochemical detector (BAS) with a glassy carbon working electrode set at 550mV vs. an Ag/AgCl reference electrode. Concentrations of 5-HT were calculated by comparing peak levels from the microdialysis samples with those of external standards of known concentrations of 5-HT. The detection limit was 0.5-1 fmol. The average of the first four or five baseline samples was taken as 100%.

5. Data analysis

5-HT levels expressed as percentages of the initial levels for each animal were analysed over the time course for each challenge by two-way analysis of variance (ANOVA), with treatment as a "between-groups" variable and time (fraction number) as a "within-groups" variable (i.e., as a repeated measure), followed by the use of planned comparisons.

Results

Basal 5-HT levels were elevated in the frontal cortex of rats, which received paroxetine either alone or together with WAY-100635, compared to the levels in rats that received saline (Table 1). A planned comparison ANOVA showed a significant overall effect of paroxetine (F [1,41] =4.08, p=0.05).

5-HT_{1A} receptor activity as measured by the effect of

Table 1. Basal levels of 5-HT in microdialysates from rats administered paroxetine and WAY-100635

Treatment	Cortex
Saline	12.4±1.9(13)
Paroxetine (5mg/kg daily for 12 days)	27.5±7.1(17)
Paroxetine+WAY-100635 (0.3mg/ml daily for 12 days)	33.4±9.3(14)

Results are expressed as femtomoles per 5 μl of dialysate, and are mean±S.E.M. of the number of observations in parentheses

8-OH-DPAT in the frontal cortex was reduced after paroxetine administration but not after the combination of paroxetine and WAY-100635. Overall analysis of the data for the action of 8-OH-DPAT in frontal cortex (Fig. 1) showed a significant effect of treatment (F [2,14]=3.55, p=0.05) and a significant effect of time after the administration of 8-OH-DPAT (F[6,84]=6.01, p=0.00003). Planned comparisons for the effect of treatment on the response to 8-OH-DPAT showed a significant difference between animals that received paroxetine and animals that received saline (F[1,14]=6.71, p= 0.021), while the other paired comparisons were not significant.

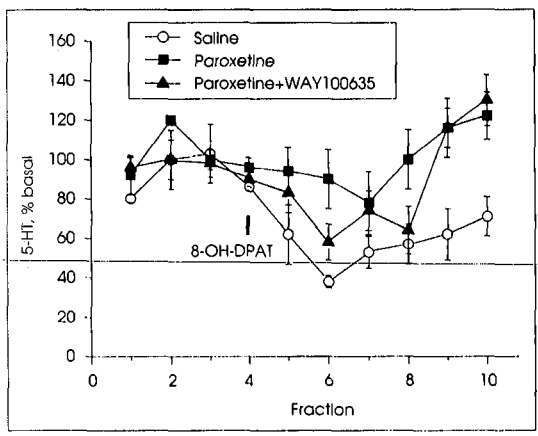

Fig. 1. Effects of paroxetine and a combination of paroxetine and WAY-100635 on the action of 8-OH-DPAT to reduce 5-HT levels in frontal cortex. Results are mean of data from five to six animals in each group.

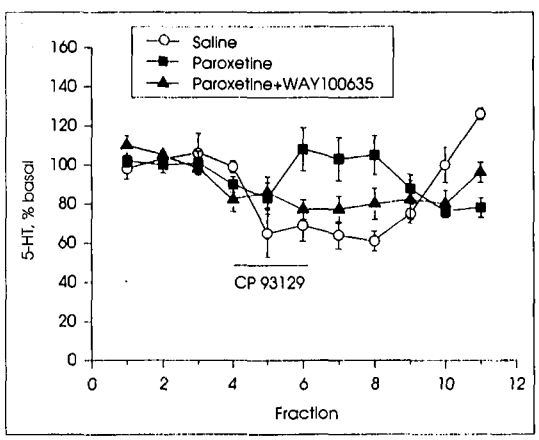

Fig. 2. Effects of paroxetine and a combination of paroxetine and WAY-100635 on 5-HT-1B autoreceptor activity in frontal cortex. Results are Mean of data from five to eight animals in each group.

Analysis of the data for 5-HT_{1B} receptor activity in the frontal cortex (Fig. 2), as measured by the effect of the 5-HT_{1B} agonist CP-93129 at 10 μM to reduce 5-HT levels, showed a significant interaction between treatment and time after administration of CP-93129 (F [14,126]=1.83, p=0.041). The response to the 5-HT_{1B} agonist was reduced in paroxetine-treated animals compared to that in animals that had received saline, while in animals that had received paroxetine and WAY-100635, an intermediate response was observed, indicating that WAY-100635 had partially prevented the paroxetine-induced desensitization of 5-HT_{1B} receptors.

Discussion

Chronic administration of paroxetine has been shown in a large number of studies, using both electrophysiological[9)10)] and in vivo microdialysis methods[11-14)] to induce subsensitivity of 5-HT_{1A} autoreceptors in the rat brain. In the majority of these studies, measurements were performed in the frontal cortex, and chronic paroxetine administration led to an increase in 5-HT release. The present results show that paroxetine, both in the presence and absence of WAY-100635, increased basal 5-HT release in the frontal cortex. Extracellular 5-HT levels are controlled by the 5-HT transporter, somatodendritic 5-HT_{1A} receptors, 5-HT_{1B} autoreceptors, and also postsynaptic 5-HT_{1A} receptors, and differential regulation of any of these by paroxetine in different brain regions may contribute to the regional differences in basal 5-HT release. Indeed, our results show that 5-HT_{1A} autoreceptor activity, as measured by the effect of 8-OH-DPAT in the frontal cortex, and 5-HT_{1B} autoreceptor activity in the frontal cortex were decreased by paroxetine

The present results showed that in addition to preventing paroxetine-induced desensitization of 5-HT_{1A} autoreceptors as measured by the effect of subcutaneous 8-OH-DPAT on 5-HT levels in the frontal cortex, WAY-100635 also prevented paroxetine-induced desensitization of 5-HT_{1B} autoreceptors in the frontal cortex. Such "heterologous" desensitization appears to represent a form of "cross-talk" between 5-HT_{1A} and 5-HT_{1B} receptors. "Cross-talk" effects have been reported in kno-

ckout mice lacking either 5-HT_{1A} or 5-HT_{1B} receptors. In 5-HT_{1A} receptor knockout mice, using in vivo microdialysis, found that the effect of the 5-HT_{1B} receptor agonist, CP 94253, to reduce 5-HT levels in striatum was actually increased, suggesting a compensatory supersensitivity of 5-HT_{1B} receptors. Clearly, the compensatory responses that occur in knockout mice, which are deficient in a particular receptor from birth, could not be expected to occur during a 12-day period of administration of a 5-HT_{1A} receptor antagonist. However, the lack of desensitization of 5-HT_{1B} receptors in rats administered the combination of paroxetine and WAY-100635 suggests that a compensatory change may have taken place at these receptors and that such a change is only evident upon increased levels of 5-HT in the synaptic gap during prolonged treatment with paroxetine.

The finding of a reduction in cortical 5-HT_{1B} autoreceptor activity after chronic administration of paroxetine provides a functional correlate for the data of[15], who found a reduction in mRNA levels coding for the 5-HT_{1B} receptor in the dorsal raphe nucleus after 7 days of daily paroxetine administration at 3mg/kg. Since no reductions were found in other brain areas, it was inferred that only 5-HT_{1B} autoreceptors and not heteroreceptors were desensitized at this time point.

Since the majority of studies show no change in 5-HT_{1A} receptor binding at either presynaptic or postsynaptic sites after prolonged selective serotonin reuptake inhibitor administration, it has been suggested that functional desensitization may be due to changes at the G-protein level[16]. showed a reduction in levels of Gi-2 and Go in the midbrain after only 3 days of paroxetine administration at 10mg/kg, and in levels of Gi-1 and Go in the hypothalamus after 7 days, and suggested that these effects may be responsible for the desensitization of somatodendritic and hypothalamic postsynaptic 5-HT_{1A} receptors, respectively. Gi and Go levels in the frontal cortex, however, were unchanged at any of the time points examined. However, in a recent abstract, found that Gi-2 levels in rat cortical membranes were significantly decreased after administration of paroxetine at 10mg/kg daily for 14 days. Gi-2 proteins have a higher coupling affinity for 5-HT_{1B} receptors than for 5-HT_{1A} receptors[17]. This finding could explain the decrease in 5-HT_{1B} autoreceptor activity in the frontal cortex observed after 12 days of paroxetine administration.

The present results, as well as those of[5], imply that concurrent treatment of patients with an SSRI and a 5-HT_{1A} receptor antagonist such as WAY-100635 would not be beneficial, since prolonged administration of the antagonist reduces or prevents the action of the selective serotonin reuptake inhibitor to desensitize 5-HT_{1A} autoreceptors rather than potentiates the effect of the selective serotonin reuptake inhibitor to increase basal 5-HT levels, as happens on acute administration. The clinical action of pindolol as an augmenting or accelerating agent in the treatment of depression must therefore have a different basis, and this view has indeed been put forward[18][19]. The only clinically relevant effect of antagonist administration appears to be its ability to induce immediate autoreceptor blockade, and an antagonist could thus be administered for a short time until the desensitizing action of the SSRI had set in, at which time antagonist treatment should be discontinued.

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