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Differential regional antagonism of 8-OH-DPAT-induced decrease in serotonin synthesis by two 5-HT_{1A} receptor antagonists

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Abstract

The effects of two 5-HT_{1A} receptor antagonists, (*R*)-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide hydrogen (2*R*,3*R*)-tartrate monohydrate (NAD-299) and *N*-(2-(1-(2-methoxyphenyl)-piperazinyl))ethyl)-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (WAY-100635) on the decrease in 5-hydroxytryptophan (5-HTP) accumulation evoked by (*RS*)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) in rats treated with the decarboxylase inhibitor, 3-hydroxyphenylhydrazine (NSD 1015) were studied in four rat brain regions: hippocampus, hypothalamus, striatum and frontal cortex. Dose-response studies revealed differential effects of both antagonists in the areas examined. Both antagonists were significantly more potent in antagonising the effect of 0.30 and 0.76 μmol/kg s.c. 8-OH-DPAT in hippocampus than in hypothalamus, striatum and frontal cortex in mentioned order. This order of potency was the opposite to that found for 8-OH-DPAT in decreasing the 5-HTP accumulation. Since previous studies by others have indicated that the reserve of somatodendritic 5-HT_{1A} receptors is greater in dorsal raphe nucleus innervating frontal cortex and striatum than in median raphe nucleus which mainly innervates hippocampus, the observed different regional potency of the two 5-HT_{1A} receptor antagonists may be explained by this difference in the 5-HT_{1A} receptor reserve. © 1998 Elsevier Science B.V.

Keywords: 5-HT_{1A} receptor; 8-OH-DPAT ((*RS*)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphtalene); Raphe; NAD-299; WAY-100635; Receptor reserve

1. Introduction

Activation of the somatodendritic 5-HT_{1A} receptors in the raphe nuclei mediates inhibition of cell firing by iontophoretic application or systemic administration of 5-HT_{1A} receptor agonists (Sprouse and Aghajanian, 1987; Blier and DeMontigny, 1987; Cox et al., 1993). Suppression of the cell firing decreases the release of 5-HT in the terminal regions measured with the in vivo microdialysis technique (Sharp et al., 1989; Auerbach et al., 1989). This also leads to decreased rates of 5-HT turnover and synthesis (Hjorth and Magnusson, 1988; Hamon et al., 1988). Meller et al. (1990) demonstrated that there is a large receptor reserve for the somatodendritic 5-HT_{1A} receptor-mediated inhibition of 5-HT synthesis in the rat cortex and hippocampus measured as the accumulation of 5-hydroxytryptophan (5-HTP) after inhibition of the 5-HTP decarboxylase. Thus, the full 5-HT_{1A} receptor agonist, 8-OH-DPAT produced maximal effect in cerebral cortex at about 20% receptor occupancy in the raphe nuclei, i.e., there is an 80% receptor reserve for the 8-OH-DPAT-induced inhibition of the synthesis of 5-HT. In hippocampus, the corresponding effect indicated a receptor reserve for 8-OH-DPAT of about 60–70% in the cell body region. Blier et al. (1990) reported an even larger difference between cortex and dorsal hippocampus in the inhibitory effect of 8-OH-DPAT on the 5-HTP accumulation.

These observations indicate that the reserve of 5-HT_{1A} receptors is different in the raphe nuclei which innervate hippocampus and cerebral cortex, respectively. Histochemical studies have shown that serotonergic neurones are derived mainly from two raphe nuclei, the dorsal and the median raphe nucleus (Dahlström and Fuxe, 1964; Steinbusch, 1981). Anterograde tracing studies showed that

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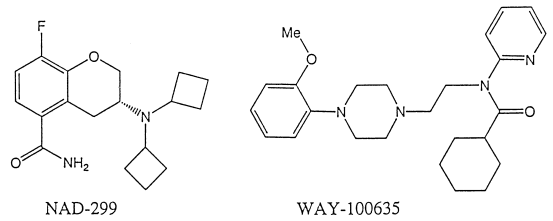

Fig. 1. Chemical structures of NAD-299 and WAY-100635 as bases.

these two raphe nuclei in part innervate different brain areas (Azmitia and Segal, 1978). Thus, the 5-HT pathways to frontal cortex and striatum are mainly derived from dorsal raphe whereas the 5-HT neurones to hippocampus emanates from both dorsal and median raphe nuclei. If these two nuclei contains different amounts of 5-HT_{1A} receptors this should influence the potencies of 5-HT_{1A} receptor antagonists to inhibit the stimulation of the somatodendritic 5-HT_{1A} receptors by endogenous 5-HT and thereby the regulation of the firing of the 5-HT neurones. In contrast to the 5-HT_{1A} receptor agonists, an antagonist of this receptor should show a potency inversely related to the receptor reserve. To test this hypothesis we have examined the effect of two selective 5-HT_{1A} receptor antagonists, (*R*)-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide hydrogen (2*R*,3*R*)-tartrate monohydrate (NAD-299) (Johansson et al., 1997) and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyrinyl)-cyclohexanecarboxamide trihydrochloride (WAY-100635) (Forster et al., 1995) (see Fig. 1 for chemical structures) in antagonising the 8-OH-DPAT-induced decrease in 5-HTP accumulation in four different rat brain regions, hippocampus, striatum, frontal cortex and hypothalamus. The 5-HTP accumulation method was chosen in this study since the rate of synthesis of 5-HT is directly related to the impulse flow (Carlsson et al., 1972; Hjorth et al., 1987), which in turn is regulated by somatodendritic 5-HT_{1A} receptors (Sprouse and Aghajanian, 1987; Blier and DeMontigny, 1987). The resulting changes in the 5-HTP accumulation can easily be measured in different brain areas of the same animal. We report here that the antagonists examined antagonised differentially the decrease in the 5-HTP accumulation in hippocampus compared to that in frontal cortex and striatum.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (B&K International, Sollentuna, Sweden), weighing 240–260 g, were housed 5 per cage under controlled temperature (21°C) and humidity with a 12 h light–dark cycle (lights on 0600 h). Food and water were freely available. The compounds were injected subcutaneously (s.c.) in a volume of 1.0 ml/kg body weight. The animal experiments were approved by the local ethics committee.

2.2. Animal treatment

The animals were injected with the test compound or saline 15 min before the administration of different doses of 8-OH-DPAT. Half an hour later the aromatic amino acid decarboxylase inhibitor, 3-hydroxybenzyl-hydrazine dihydrochloride (NSD 1015) (pH adjusted to 6 with NaOH), 475 μmol/kg s.c., was injected and the rats were killed 30 min thereafter. The brain regions examined were rapidly dissected and frozen on dry ice. They were stored at −70°C until analysis.

2.3. Analysis of 5-HTP

The concentration of 5-HTP in the different brain regions was determined by high performance liquid chromatography with electrochemical detection according to the method of Magnusson et al. (1980). The mobile phase was 0.1 M phosphate buffer (pH 2.5):methanol:acetonitrile (89:9:2, v/v), containing 1 mM octylsulphate. The frozen samples were weighed and homogenised in 10 vol (w/v) 0.1 M perchloric acid, containing 2.5 mM sodium bisulphite, 1 mM ethylendiamine tetra acetic acid (EDTA) and isoprenaline as internal standard. After centrifugation ($14\,000 \times g$ for 10 min at 4°C) the supernatant (50 μl) was injected directly onto a Supelcosil LC-18-DB (3 μm) column, connected to a detector (ESA Coulochem 5100A), set to 0.05/0.30 V. The retention time for 5-HTP was 15 min and the detection limit was 0.1 pmol.

2.4. Statistical evaluation

Mean values were compared with Dunnett's *t*-test following a one-way analysis of variance (ANOVA). Dose-response curves were compared with a factorial two-way ANOVA program (StatView 4.02). A P-value < 0.05 was regarded as significant.

2.5. Compounds

NAD-299 ((*R*)-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide hydrogen (2*R*,3*R*)-tartrate monohydrate) and WAY-100635 (*N*-(2-1-methoxyphenyl)-piperazinyl)ethyl)-*N*-pyridinyl)cyclohexanecarboxamide trihydrochloride were provided by Astra Arcus. 8-OH-DPAT ((*RS*)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphtalene hydrobromide)) was bought from RBI Research Biochemical, Natick, MA, USA. 3-Hydroxybenzylhydrazine dihydrochloride (NSD 1015) was purchased from Sigma, St. Louis, MO, USA. Other compounds used were of highest purity available.

3. Results

3.1. The accumulation of 5-HTP in four brain regions

The amount of 5-HTP accumulated in hippocampus, hypothalamus, striatum and frontal cortex 30 min after the injection of 475 μmol/kg s.c. NSD1015 is shown in Table 1. The rate of 5-HT synthesis was twice as rapid in hypothalamus as in the other regions.

3.2. Dose-response curves of the decrease in 5-HTP accumulation by 8-OH-DPAT

The dose-response curves of the decrease in 5-HTP accumulation by 8-OH-DPAT in the four brain regions studied are shown in Fig. 2. The maximal inhibition by 8-OH-DPAT reached approximately 50% in the four regions studied. The potency of 8-OH-DPAT was highest in frontal cortex followed by striatum hypothalamus and hippocampus. A two-way ANOVA showed significant difference between frontal cortex and hippocampus ($F(1,46) = 9.002$; $P = 0.0043$), but not between the other regions analysed.

3.3. Effect of NAD-299 and WAY-100635 on the 5-HTP accumulation

Neither NAD-299, 0.9 μmol/kg s.c. nor WAY-100635, 0.3 μmol/kg s.c. had any effect upon the 5-HTP accumulation in the brain regions studied when compared with the saline-treated controls, in accordance with previous results (Johansson et al., 1997).

3.4. Inhibition of the 8-OH-DPAT-induced decrease in the 5-HTP accumulation by NAD-299

When the animals were pre-treated with 0.9 μmol/kg s.c. NAD-299, 15 min before the administration of different doses of 8-OH-DPAT the dose-response curves of the decrease in the 5-HTP accumulation were shifted to the right with a factor of 10–20 (Fig. 2, Table 2). This indicates competition between NAD-299 and 8-OH-DPAT. The regional effects of 8-OH-DPAT in the presence of NAD-299 was similar to that in the saline-treated animals

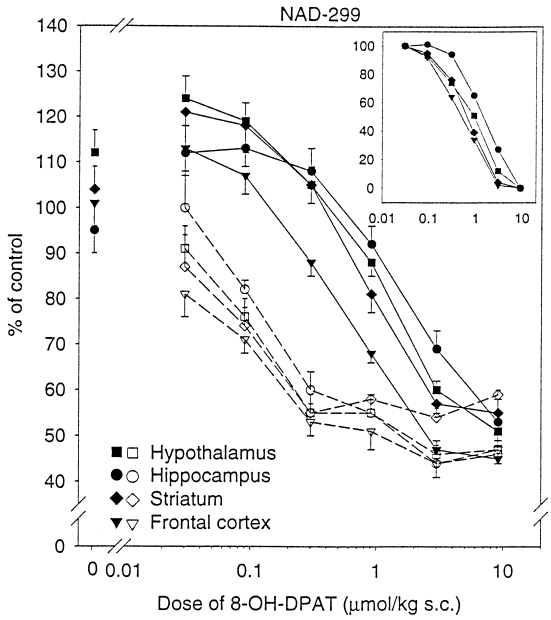

Fig. 2. The effect of NAD-299 on the 8-OH-DPAT-induced decrease in the 5-HTP accumulation in four different brain regions. Saline (open symbols), NAD-299 (0.9 μmol/kg s.c. = 0.4 mg/kg) (closed symbols) were injected 15 min before saline or different doses of 8-OH-DPAT and 45 min before NSD1015, 475 μmol/kg s.c. (100 mg/kg). The effect of NAD-299 itself on the 5-HTP accumulation compared with the saline control is shown to the left. The rats were killed 30 min later and the brain regions, hypothalamus, hippocampus, striatum and frontal cortex were dissected, frozen on dry ice and stored at −70°C until analysis. Values are means of 5 (saline controls) or 10 (NAD-299 treated) rats with S.E.M. shown as vertical bars. Inset: normalised dose-response curves of the NAD-299 pretreated rats in which the effect at 0.03 and 10 μmol/kg is set to 100 and 0%, respectively.

but the difference between frontal cortex and other regions was even more accentuated. Thus, the dose-response curves of the inhibition of the 5-HTP accumulation by 8-OH-DPAT showed that NAD-299 produced significantly weaker antagonism in the frontal cortex than in the other brain regions when analysed with a factorial two-way ANOVA (vs. striatum $F(1,106) = 42.404$, $P < 0.0001$; vs. hypothalamus $F(1,107) = 60.267$, $P < 0.0001$; vs. hippocampus $F(1,107) = 48.570$, $P < 0.0001$). No difference between the other three regions was observed. However, a

Table 1
Accumulation of 5-HTP in saline treated control rats, killed 30 min after injection of 475 μmol/kg s.c. (100 mg/kg) NSD 1015

Brain region	5-HTP, nmol/g tissue
Hypothalamus	1.031 ± 0.027
Hippocampus	0.489 ± 0.007
Striatum	0.466 ± 0.010
Frontal cortex	0.404 ± 0.006

Values are means ± S.E.M. from 40 rats.

Table 2
ED_{50} values for 8-OH-DPAT in decreasing the 5-HTP accumulation in the different brain regions studied, estimated from the experiments shown in Figs. 2 and 3 by four-parametric logistic fits

Treatment	ED_{50}, μmol/kg s.c.			
	Hippocampus	Hypothalamus	Striatum	Frontal cortex
Saline	0.13	0.09	0.09	0.13[a]
NAD-299	1.73	0.97	0.69	0.50
WAY-100635	3.26	0.98	1.08	0.92

The dose of NAD-299 and WAY-100635 were 0.9 μmol/kg s.c. (0.44 mg/kg) and 0.3 μmol/kg s.c. (0.17 mg/kg), respectively.
[a]Obvious to high value because of missing low doses; a value of 0.07 would be more correct.

low dose (0.03 μmol/kg) of 8-OH-DPAT combined with 0.9 μmol/kg NAD-299 tended to increase the rate of the 5-HTP accumulation compared to the saline- and NAD-299-treated control groups (Fig. 2). Since this enhancement of the 5-HTP accumulation was somewhat different between the regions and thereby may affect the whole dose-response curves, the insert to Fig. 2 shows the corresponding curves in which the values at 0.03 and 10 μmol/kg 8-OH-DPAT were set to 100 and 0%, respectively. By this normalisation, the curves for frontal cortex and striatum become more closer together.

3.5. Inhibition of the 8-OH-DPAT-induced decrease in 5-HTP accumulation by WAY-100635

As shown in Fig. 3 and Table 2, WAY-100635 at the dose 0.3 μmol/kg s.c. markedly inhibited the 8-OH-DPAT-induced decrease in the 5-HTP accumulation in a competitive manner. The effect was similar to that observed for NAD-299 with the exception that no significant difference between the brain areas was obtained. Also in this experiment there was a tendency of enhanced 5-HTP accumulation at the lowest dose (0.1 μmol/kg) 8-OH-DPAT tested in combination with WAY-100635. The insert of Fig. 3 shows the dose-response curves when the enhancement of the 5-HTP accumulation is taken into account.

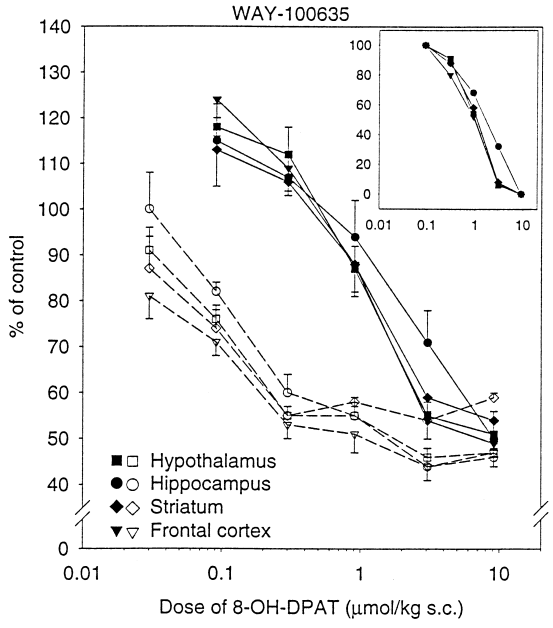

Fig. 3. The effect of WAY-100635 on the 8-OH-DPAT-induced decrease in the 5-HTP accumulation in four different brain regions. Saline (the same experiment as in Fig. 2) (open symbols), WAY-100635 (0.3 μmol/kg s.c. = 0.17 mg/kg) (closed symbols) were injected 15 min before saline or different doses of 8-OH-DPAT and 45 min before NSD1015, 475 μmol/kg s.c. (100 mg/kg). The rats were killed 30 min later and the brain regions, hypothalamus, hippocampus, striatum and frontal cortex were dissected, frozen on dry ice and stored at −70°C until analysis. Values are means of 5 rats with S.E.M. shown as vertical bars. Insert: normalised dose-response curves of the WAY-100635 pretreated rats in which the effect at 0.1 and 10 μmol/kg is set to 100 and 0%, respectively.

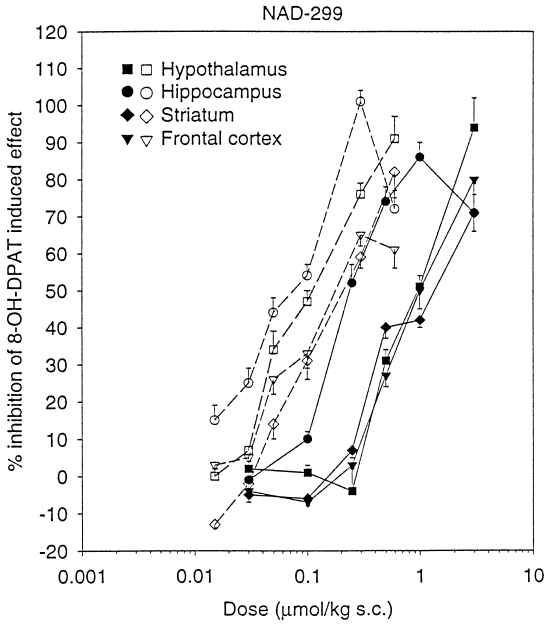

Fig. 4. Dose-response curves of the antagonistic effect of NAD-299 on the decrease in 5-HTP accumulation evoked by two different doses of 8-OH-DPAT, 0.3 μmol/kg s.c. (0.1 mg/kg) (open symbols) and 0.76 μmol/kg s.c. (0.25 mg/kg) (closed symbols) in hypothalamus, hippocampus, striatum and frontal cortex. Saline or different doses of NAD-299 were injected 15 min before 8-OH-DPAT and 45 min before NSD 1015, 475 μmol/kg s.c. (100 mg/kg). The rats were killed 30 min after the NSD 1015 injection, the brain regions were dissected, frozen on dry ice and stored at −70°C until analysis. Values are means of 5 rats with S.E.M. shown as vertical bars. The antagonism is expressed in percentage of the decrease in the 5-HTP accumulation evoked by 8-OH-DPAT.

3.6. Dose-response curves of NAD-299 at fixed doses of 8-OH-DPAT

The decrease in 5-HTP accumulation by 8-OH-DPAT, 0.3 μmol/kg s.c., was dose-dependently antagonised by NAD-299 between 0.015 and 3 μmol/kg s.c. (Fig. 4). The effect measured in hippocampus was obtained at significantly lower doses compared to striatum ($F(1,48) = 18.85$; $P < 0.0001$), frontal cortex ($F(1,48) = 14.623$; $P = 0.0004$) but not hypothalamus ($F(1,48) = 3.008$; $P = 0.0894$). The ED_{50} values of the antagonism estimated from these curves are given in Table 3. At higher dose of 8-OH-DPAT (0.76 μmol/kg s.c.), the dose-response curves shifted to the right indicating competition between 8-OH-DPAT and NAD-299. The potency of the antagonism in hippocampus was significantly greater than in striatum ($F(1,48) = 23.207$; $P < 0.0001$), frontal cortex ($F(1,48) = 22.910$; $P = < 0.0001$) and hypothalamus ($F(1,48) = 10.608$; $P = 0.021$. No difference between the latter three regions was obtained.

Table 3
ED_{50} values for NAD-299 and WAY-100635 in antagonising the decrease in 5-HTP accumulation induced by 8-OH-DPAT, 0.3 μmol/kg s.c. (0.1 mg/kg) and 0.76 μmol/kg s.c. (0.25 mg/kg), estimated from the experiments shown in Figs. 4 and 5 by four-parametric logistic fits

ED_{50} (μmol/kg s.c.)	Dose 8-OH-DPAT (μmol/kg s.c.)	
	0.3	0.76
NAD-299		
Hippocampus	0.07	0.20
Hypothalamus	0.08	0.94
Striatum	0.10	0.56
Frontal cortex	0.08	0.75
WAY-100635		
Hippocampus	0.016	0.05
Hypothalamus	0.027	0.08
Striatum	0.021	0.27
Frontal cortex	0.023	0.29

3.7. Dose-response curves of WAY-100635 at fixed doses of 8-OH-DPAT

The dose-response of the antagonism by WAY-100635 of the 8-OH-DPAT-induced decrease in 5-HTP accumulation was examined at the same doses of 8-OH-DPAT as used for NAD-299 (Fig. 5, Table 3). The pattern of the antagonism in the four regions examined was the same as that observed for NAD-299. At both doses of 8-OH-DPAT (0.3 and 0.76 μmol/kg s.c.) WAY-100635 was most potent in antagonising the effect of 8-OH-DPAT in hippocampus followed by hypothalamus, striatum and frontal cortex. At 0.3 μmol/kg 8-OH-DPAT there was no significant difference between hippocampus and hypothalamus ($F(1,67) = 1.552$; $P = 0.2172$). However, hippocampus differed significantly from striatum ($F(1,67) = 10.273$; $P = 0.0021$) and frontal cortex ($F(1,66) = 13.756$; $P = 0.0004$). At the higher dose, there were statistically significant differences of the antagonism by WAY-100635 in hippocampus compared with hypothalamus ($F(1,108) = 5.178$; $P = 0.0248$), striatum ($F(1,108) = 27.959$; $P < 0.0001$) and frontal cortex ($F(1,108) = 53.179$; $P < 0.0001$). The antagonism in hypothalamus was significantly different from that in striatum ($F(1,108) = 15.101$; $P = 0.0002$) and frontal cortex ($F(1,108) = 42.035$; $P < 0.0001$). No statistical difference between striatum and frontal cortex was obtained.

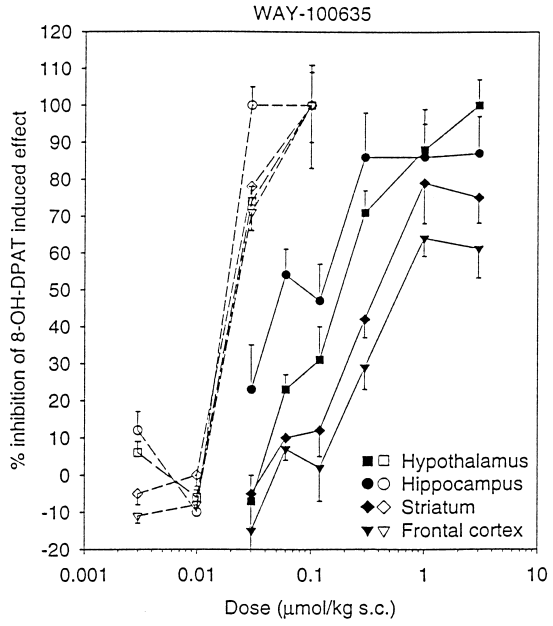

Fig. 5. Dose-response curves of the antagonistic effect of WAY-100635 on the decrease in 5-HTP accumulation evoked by two different doses of 8-OH-DPAT, 0.3 μmol/kg s.c. (0.1 mg/kg) (open symbols) and 0.76 μmol/kg s.c. (0.25 mg/kg) (closed symbols) in hypothalamus, hippocampus, striatum and frontal cortex. Saline or different doses of WAY-100635 were injected 15 min before 8-OH-DPAT and 45 min before NSD 1015, 475 μmol/kg s.c. (100 mg/kg) The rats were killed 30 min after the NSD 1015 injection, the brain regions were dissected, frozen on dry ice and stored at −70°C until analysis. Values are means of 5 to 10 rats with S.E.M. shown as vertical bars. The antagonism is expressed in percentage of the decrease in the 5-HTP accumulation evoked by 8-OH-DPAT.

4. Discussion

It is well established that the rate of 5-HT synthesis measured as the 5-HTP accumulation when the 5-HTP decarboxylase is inhibited is modulated by the impulse flow in the serotonergic neurones (Carlsson et al., 1972; Hjorth et al., 1987; Meller et al., 1990). It is also clearly documented from electrophysiological studies that the firing rate in these neurones is negatively regulated by somatodendritic 5-HT_{1A} receptors in the raphe nuclei (Sprouse and Aghajanian, 1987; Blier and DeMontigny, 1987). Thus, activation of the somatodendritic 5-HT_{1A} receptors results in a decrease in the impulse flow and thereby also in the synthesis and release of 5-HT in the nerve terminal regions. Measuring changes in the 5-HT synthesis with the 5-HTP accumulation technique is accordingly a convenient method to examine the action of a 5-HT_{1A} receptor agonist, e.g., 8-OH-DPAT on the somatodendritic receptors, especially if the effect in different terminal regions has to be compared.

The 5-HT-containing neurones in the rat brain are derived from the raphe nuclei in midbrain (Dahlström and Fuxe, 1964; Steinbusch, 1981). Although some mixed input exists, the dorsal raphe nucleus mainly innervates striatum and frontal cortex whereas the terminals in hippocampus emanate from both median and dorsal raphe nuclei (Azmitia and Segal, 1978). There are some controversies upon the contribution of the two nuclei in innervating the hippocampal serotonergic system. Kreiss and Lucki (1994) using local application of 8-OH-DPAT in dorsal and median raphe nuclei and measuring the release of 5-HT in striatum and ventral hippocampus with in vivo microdialysis technique found a complete separation between these systems in that application of 8-OH-DPAT

into the dorsal raphe nucleus reduced 5-HT release in striatum but not in ventral hippocampus. Conversely, administration of 8-OH-DPAT in median raphe nucleus reduced 5-HT release in ventral hippocampus but not in striatum. Furthermore, local application of the receptor antagonist (−)-propranolol into these nuclei antagonised the effect of systemic injected 8-OH-DPAT with the same pattern as that found after local application of 8-OH-DPAT. These observations indicate that the release of 5-HT in these two regions is regulated from different raphe nuclei. McQuade and Sharp (1995), on the other hand, found that electrical stimulation of dorsal or median raphe nucleus with implanted electrodes produced a similar increase in extracellular 5-HT in ventro-medial hippocampus recorded with microdialysis, whereas the contribution of the median raphe nucleus to the release of 5-HT in frontal cortex was much less than that of dorsal nucleus. These controversial findings may in part be explained by different contribution of the innervation from dorsal raphe in various parts of hippocampus (Azmitia and Segal, 1978; Köhler and Steinbusch, 1982). Thus, in dentate gyrus the 5-HT pathway seems to be entirely innervated from median raphe.

Since the whole hippocampus was analysed in the present study, it is likely that some contribution of 5-HT terminals derived from dorsal raphe nucleus, but the main part of the 5-HT nerve terminals emanates from median nucleus in this region. The main part of the serotonergic neurones in striatum and frontal cortex are derived from dorsal raphe nucleus whereas hypothalamus is innervated from both nuclei (Azmitia and Segal, 1978).

Meller et al. (1990) obtained evidence, using the irreversibly acting receptor inactivator *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline hydrochloride (EEDQ) and the 5-HTP accumulation technique, that the 5-HT_{1A} receptor reserve at those receptors regulating 5-HT synthesis is larger in cortex than in hippocampus. Our findings that the potency of 8-OH-DPAT was significantly 2-fold greater in frontal cortex than in hippocampus agree with the results of these authors. Although small, this difference appears to be robust since it has been repeatedly observed in dose-response studies with other 5-HT_{1A} receptor agonists, e.g., flesinoxan and buspirone (unpublished observations). The difference between dorsal and median raphe nuclei was even more pronounced in the study of Sinton and Fallon (1988) in which the decrease in the firing rate by 8-OH-DPAT of 5-HT cells in the two nuclei was recorded. 8-OH-DPAT was almost 30 times more potent in dorsal than medial raphe. Similar results have been obtained by Blier et al. (1990).

The main new finding in the present study was that the two 5-HT_{1A} receptor antagonists studied, NAD-299 and WAY-100635, showed regional differences in their potencies to antagonise the 8-OH-DPAT-induced decrease in the 5-HTP accumulation. The order of potency was hippocampus > hypothalamus > striatum = frontal cortex, i.e., the opposite to that found for the 5-HT_{1A} receptor agonist, 8-OH-DPAT. This order of potency might be explained by the difference in the 5-HT_{1A} receptor reserves; the greater the reserve the higher the required dose of the antagonist to give a wanted effect, since more receptors have to be blocked to prevent the larger stimulus imparted by a particular dose of agonist. This difference may be important in studies of the functional effects of 5-HT_{1A} receptor antagonists, particularly when combined with 5-HT uptake inhibitors (Artigas, 1993), since hippocampus requires two to six times less amount of the antagonist than frontal cortex for similar degree of inhibition (Table 3).

The very steep dose-response curves for WAY-100635 in antagonising the inhibitory effect of the low dose (0.1 mg/kg) 8-OH-DPAT on the 5-HTP accumulation compared with the corresponding curves for NAD-299 (Figs. 4 and 5) cannot be satisfactory explained with the data available to date. A rapid metabolism of WAY-100635 at the low dose might have contributed to the steep dose-response curves.

In the dose-response study of 8-OH-DPAT-induced decrease in the 5-HTP accumulation NAD-299 (Fig. 2), but not WAY-100635 (Fig. 3), was an apparently weaker antagonist in frontal cortex than in the other regions. However, this finding may be an artefact due to regional differences in the enhancement of the 5-HTP accumulation at low doses of 8-OH-DPAT in combination with NAD-299. When these differences were taken into account the curves for frontal cortex and striatum come closer together (Fig. 2, inset). This is also in accordance with the results obtained in the experiment with NAD-299 at two fixed doses of 8-OH-DPAT (Fig. 4) in which no difference between frontal cortex and striatum was observed, in agreement with a mainly dorsal raphe origin of the serotonergic neurones to these brain regions.

The clear tendency of the enhancement of 5-HTP accumulation at low doses of 8-OH-DPAT when the 5-HT_{1A} receptors were blocked by NAD-299 or WAY-100635 is interesting although we have no good explanation of the phenomenon. One possibility is that the 5-HT_{1A} receptor blockade is unmasking a stimulatory effect of 8-OH-DPAT on the 5-HT neurotransmission. Further studies with other 5-HT_{1A} receptor agonists will reveal whether this effect is common to all 5-HT_{1A} receptor agonists.

In conclusion, this study shows that the two 5-HT_{1A} receptor antagonists, NAD-299 and WAY-100635 which have quite different chemical structures are significantly more potent in antagonising the 8-OH-DPAT-induced decrease in 5-HTP accumulation in hippocampus than in frontal cortex and striatum whereas the potency in hypothalamus appears to be intermediate. A possible explanation of these findings is that the reserve of the 5-HT_{1A} receptors is less in median raphe nucleus from which hippocampus is mainly innervated than in dorsal raphe nucleus from which the main 5-HT neurones to frontal cortex and striatum originates. Hypothalamus is innervated from both nuclei and showed an intermediate sensitivity.

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