ELSEVIER

European Journal of Pharmacology 307 (1996) 211–217

ejp

Genomic responses to 5-HT_{1A} or 5-$HT_{2A/2C}$ receptor activation is differentially regulated in four regions of rat brain

Nanda Tilakaratne, Eitan Friedman *

Division of Molecular Pharmacology, Departments of Pharmacology and Psychiatry, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129, USA

Received 12 February 1996; accepted 15 March 1996

Abstract

The functional profiles of brain 5-HT_{1A} and 5-$HT_{2A/C}$ receptors were assessed by quantitating changes in the immediate early genes — *c-fos*, *ngf1c* and *tis1*, following receptor activation with either 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) or DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane). Stimulation of either class of 5-HT receptor elicited an induction of all three immediate early genes to varying extents in cortex, hippocampus and cerebellum, but not in striatum. The responses to 8-OH-DPAT peaked earlier than those to DOI. WAY 100135 (*N*-tertiobutyl-3-[4-(2-methoxyphenyl)-piperazinyl]-2-phenylpropanamide), the putative 5-HT_{1A} receptor antagonist blocked the 8-OH-DPAT effect but not the responses to DOI. WAY 100135 by itself also elicited a relatively smaller genomic response. Ketanserin completely abolished the DOI-induced genomic responses. The results support the earlier findings that 5-HT_{1A} receptor sites are abundant in frontal cortex and hippocampus. In addition, the robust genomic responses to 8-OH-DPAT as well as Northern hybridization with a cDNA probe for 5-HT_{1A} mRNA in the cerebellum clearly implicate the functional expression of 5-HT_{1A} receptors in this brain region. The responses to the 5-HT_2 receptor agonist, DOI support a greater abundance of these receptors in the cortex, and relatively lower levels in hippocampus and cerebellum. The results suggest a differential induction pattern among the three immediate-early genes depending on the brain region and the 5-HT receptor subtype involved.

Keywords: 5-HT_{1A} receptor; 5-$HT_{2A/C}$ receptor; *c-fos*; *ngf1c*; *tis1*

1. Introduction

Serotonin (5-HT [5-hydroxytryptamine]) exerts its effects through several different receptor subtypes, each of which is coded for by a distinct gene, and possess distinct pharmacological properties and physiological functions (Hoyer et al., 1994). Of the many receptor subtypes, the 5-HT_{1A} and 5-$HT_{2A/C}$ receptors have been most extensively characterized, largely due to the availability of specific agonists for these sites. 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) and DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) selective agonists for the 5-HT_{1A} and 5-$HT_{2A/C}$ receptors respectively, and ketanserin is a selective 5-$HT_{2A/C}$ antagonist. No effective 5-HT_{1A} selective antagonist has been available until recently when the phenyl piperazine compound, WAY 100135 (*N*-tertiobutyl-3-[4-(2-methoxyphenyl)-piperazinyl]-2-phenylpropanamide) was shown to meet that need (Routledge et al., 1993; Fletcher et al., 1993). Selective ligand binding studies and in situ hybridization which map the respective mRNA's have shown that the 5-HT receptor subtypes are differentially localized in the mammalian central nervous system (Palacios et al., 1990).

Stimulation of neurotransmitter receptors results in a complex series of events which converge on the nucleus to elicit a rapid increase in the transcription of several genes collectively called immediate early genes (Dragunow et al., 1989). Among the multitude of immediate early genes that have been described are *c-fos* and *c-jun* which are the cellular counterparts of viral oncogenes (Sagar et al., 1988). *ngf1a, 1b* and *1c* which were originally identified as those induced by nerve growth factor (Morgan and Curran, 1991), and a family of genes (*tis1, 7, 8, 10* and *21*) induced by the phorbol ester TPA (12-*O*-tetradecanoylphorbol 13-acetate), hence called TPA inducible sequences, or *tis* (Lim et al., 1987). The protein products

* Corresponding author. Division of Molecular Pharmacology, Department of Pharmacology, Medical College of Pennsylvania/EPPI, 3200 Henry Avenue, Philadelphia, PA 19129, USA. Tel.: +1 215 8424203; fax: +1 215 8431515.

Elsevier Science B.V.
PII S0014-2999(96)00233-6

of these genes function as transcription factors to modulate the expression of other genes, generally referred to as 'late effector genes'. The activation of immediate-early genes constitutes one of the primary steps of the mechanism by which stimuli at the cell membrane are transduced into short and long-term responses to neuronal activation (Sheng and Greenberg, 1990). Furthermore, there is evidence that not all immediate-early genes respond uniformly to a given stimulus, but there is differential response, depending on the nature of the receptor or the stimulus. This supports the suggestion that distinct combinations of genes could confer specificity in cellular response to different stimuli.

The basal expression of most immediate early genes is minimal. As such, the rapid and robust increase in response to a stimulus is easily detected. For these reasons, assay for the induction of immediate early gene expression has come to be regarded as a useful tool to map neuronal activation. In the present study, 5-HT_{1A} or 5-$HT_{2A/C}$ receptor agonist-induced immediate early gene expression was used to profile the distribution of the receptor subtypes, and their activation patterns, in four different regions of rat brain. Three different immediate-early genes (*c-fos, ngfic and tis1*) were included in the study to determine whether the genomic response to 5-HT receptor stimulation is uniform or different across the different brain regions. The results indicate a spatial and temporal pattern of differential gene expression depending on the receptor subtype, brain region or the immediate early gene involved.

2. Materials and methods

2.1. Animals and treatments

Male Sprague-Dawley rats (200–225 g) were used. They were maintained on a 12 h light-dark cycle, with a constant supply of food and drinking water.

Ketanserin, 8-OH-DPAT and DOI-HCl were purchased from Research Biochemicals International, MA. WAY 100135 was a gift from Wyeth Research Ltd., Berkshire, U.K.

On the day of the experiment, the rats were moved to a different room, allowed to acclimate for 1 h, and were rapidly injected i.p. with either the selected drug or saline according to the following regime: a single injection of 8-OH-DPAT (4 mg/kg in 0.3 ml saline); DOI (4 mg/kg in 0.3 ml saline), or 0.3 ml saline. The choice of the dose of each agonist or antagonist was based on existing data in the literature. In testing the effects of antagonist-agonist combinations, each rat was administered two injections, 20 min apart, according to one of the following regimes: (a) ketanserin (4 mg/kg in 0.3 ml saline) followed with 8-OH-DPAT, (b) ketanserin followed with DOI, (c) WAY 100135 (10 mg/kg in 0.3 ml of saline, subcutaneous) followed with 8-OH-DPAT, (d) WAY 100135 followed with DOI, (e) saline followed with 8-OH-DPAT, (f) saline followed with DOI, (g) ketanserin followed with saline, (h) WAY 100135 followed with saline, and (i) saline followed with saline. Rats were sacrificed by rapid decapitation, at times corresponding to either 30 or 90 min after the injection of the 5-HT receptor agonist or saline (control). The rats were rapidly decapitated and the 4 brain regions, frontal cortex, hippocampus, striatum and cerebellum were dissected and immediately frozen on dry ice until they were processed for extraction of RNA, about 2 h later. Four rats were included in each treatment regime/time point group.

2.2. RNA Extraction and analysis

Total RNA from the individual brain regions was extracted by the guanidine isothiocyanate method (Chomczynski and Sacchi, 1987). Ten μg of each sample of RNA was size fractionated by electrophoresis on 1% agarose; 6% formaldehyde gel. The uniformity of loading of the samples was assessed based on ethidium bromide staining. Only those gels satisfying this condition were used in blotting and Northern analysis. RNA on the gel were transferred onto a nylon based membrane (Nytran: Schleicher and Schuell) by capillary transfer, and fixed by exposure to UV irradiation (Stratagene, CA) for 2 min. Following a 2 h prehybridization at 42°C in 50% formamide, 5 × SSC (1 × SSC = 0.3 M NaCl, 0.03 M Na citrate); 5 × Denhardt's solution (1 × Denhardt's = 0.02% bovine serum albumin, 0.2% polyvinyl pyrillidone, 0.2% Ficoll) and 0.1% sodium dodecyl sulfate, hybridization was done in the same solution together with 10% dextran sulfate, denatured salmon sperm DNA (100 μg/ml) and 10×10^6 cpm of total activity of appropriate ^{32}P-labeled probe. Hybridized membrane was washed once at room temperature for 20 min in 2 × SSC; 0.1% SDS followed by a second wash at 55°C in 0.2 × SSC; 0.1% SDS. Membrane was exposed to X-ray film (Fuji) with an intensifying screen at −70°C for 3 to 5 days. The relevant signals on the autoradiogram were quantitated by laser scan densitometry (Zeineh, Biomed Instruments, CA).

The initial Northern hybridization analyses established that each of the probes that was used hybridized to a single species of mRNA. Therefore, in the subsequent experiments involving the combined antagonist-agonist treatments, the RNA was analyzed by dot-blot hybridization, using a dot-blot apparatus (Schleicher-Schuell) according to the method described by Davis et al. (1986). The hybridization conditions employed were similar to those described above.

2.3. Probes

A 500 bp, Apa I restriction fragment of the human *c-fos* cDNA was used as the probe for detecting *c-fos* mRNA. Similarly, *tis1* and *ngf1c* mRNA were detected using 500

or 450 bp restriction fragments of the respective cDNA's (kindly supplied by Dr. D. Filer of New York University Medical Center). The probe for the 5-HT_{1A} receptor was a 900 bp *Bam* HI-*PSt* I restriction fragment of the rat cDNA (kindly provided by Dr. Olivier Civelli, Oregon Health Science Center), while the 5-HT_2 receptor probe was a 1100 bp BamHI restriction fragment of rat cDNA (kindly provided by Dr. D. Pritchett of the University of Pennsylvania). Probes were prepared by radio-labeling the cDNA fragments by the random primer labeling method.

3. Results

3.1. Effect of 8-OH-DPAT or DOI on immediate early gene expression

Representative Northern blots which show the effects of 8-OH-DPAT or DOI on the expression of *c-fos*, *tis1* and *ngf1c* mRNA in four different brain regions, at 30 or 90 min post treatment, are presented in Fig. 1. The mean percent quantitative change in each of the three species of mRNA in the cortex, hippocampus and cerebellum, in response to 8-OH-DPAT, expressed as percent stimulation above the mRNA levels in saline injected control rats are presented in Fig. 2. A highly significant ($P < 0.05$) stimulation of *c-fos* and *ngf1c* gene expression was evident in the cortex (198–221%), hippocampus (259–333%) and in the cerebellum (188–274%), at 30 min post treatment. The response was either sustained (hippocampus), or slightly reduced (cortex and cerebellum) at 90 min post-treatment. No significant response was seen in the striatum at either time point. Stimulation of *tis1* gene expression, on the other hand, was comparatively lower, yet significant ($P < 0.05$) in cortex (182%), hippocampus (83%) and cerebellum (109%).

Stimulation of the expression of the three genes at 30 min following DOI treatment was much weaker (ranging from 49–157%), when compared with that following 8-OH-DPAT (Figs. 1 and 3). Acute injection of DOI resulted in a dramatic stimulation of *c-fos*, at 90 min post-injection, in the cortex (327%), hippocampus (281%) and in the cerebellum (190%), each of which was highly significant ($P < 0.05$). A stimulation of similar magnitude was also seen in *ngf1c* gene expression; cortex (290%), hippocampus (233%) and cerebellum (190%). A significant *tis1* gene expression following DOI, however, was limited to the cortex (219%; $P < 0.05$) and cerebellum (125%; $P < 0.05$), only. None of the three genes was induced to a significant level in the striatum after DOI treatment.

3.2. Effect of pretreatment with either WAY 100135 or ketanserin on the immediate early gene responses to 8-OH-DPAT

The mean quantitative changes of the three immediate early gene mRNA's in response to the combined treatment of WAY 100135 followed 20 min later with 8-OH-DPAT, relative to that in the saline-saline control group, are presented in Fig. 2. Prior treatment with WAY 100135 resulted in a decrease in the 8-OH-DPAT induced stimulation of all three genes; *c-fos* (reduced from 221 ± 22 to 102 ± 24, 259 ± 16 to 91 ± 15, and 188 ± 17 to 65 ± 11% in cortex, hippocampus and cerebellum, respectively), *ngf1c* (reduced from 198 ± 12 to 62 ± 14, 333 ± 16 to 93 ± 10 and from 224 ± 36 to 69 ± 15 percent in cortex, hippocampus and cerebellum, respectively) and *tis1* (reduced from 157 ± 8 to 46 ± 14 and 109 ± 7 to 52 ± 8 percent in cortex and cerebellum, respectively). The difference between group mean ± SE of 8-OH-DPAT alone and WAY 100135 + 8-OH-DPAT in each case was statistically significant ($p < 0.05$) as determined by ANOVA followed by Dunnett's multiple range test. The trend was similar at 90 min post-injection. Ketanserin treatment, on the other hand, did not change the response to 8-OH-DPAT. Rats injected with WAY 100135 followed by saline showed a small (mean of 57%) but significant ($P < 0.05$) increase in the immediate early gene expression in the cortex and hippocampus. In the cortex, all three genes were stimulated ($P < 0.05$) by WAY 100135, whereas in the hippocampus, it was limited to *c-fos* only ($P < 0.05$).

3.3. Effect of pretreatment with either WAY 100135 or ketanserin on the immediate early gene responses to DOI

Fig. 3 presents the mean quantitative changes in *c-fos*, *tis1* and *ngf1c* mRNAs following DOI with or without prior treatment with ketanserin, expressed as percent stimulation over that of the saline-saline controls. Prior treatment with ketanserin completely abolished the DOI induced stimulation of all 3 genes in the cortex, hippocampus and cerebellum. The 5-HT_{1A} antagonist, WAY 100135 did not alter the expression of *c-fos* and *tis1* and *ngf1c* in response to DOI. Ketanserin, by itself, failed to induce the expression of these three genes in any of the brain regions that were examined.

3.4. Expression of the 5-HT_{1A} and 5-HT_2 receptor mRNA in four brain regions

Northern analyses to detect 5-HT_{1A} and 5-HT_{2A} receptor mRNA in rat frontal cortex, hippocampus, cerebellum and striatum were performed (data not shown). The 5-HT_{1A} cDNA probe bound to a mRNA of approximately 4.0 kb, strongly in cortex, hippocampus and cerebellum, and weakly in the striatum. An additional weak band of approximately 3.3 kb was also detected in cortex, hippocampus and cerebellum. The 5-HT_{2A} receptor probe hybridized to a 5.2–5.5 kb (approximate size) message in cortex, hippocampus and cerebellum, but not in the striatum.

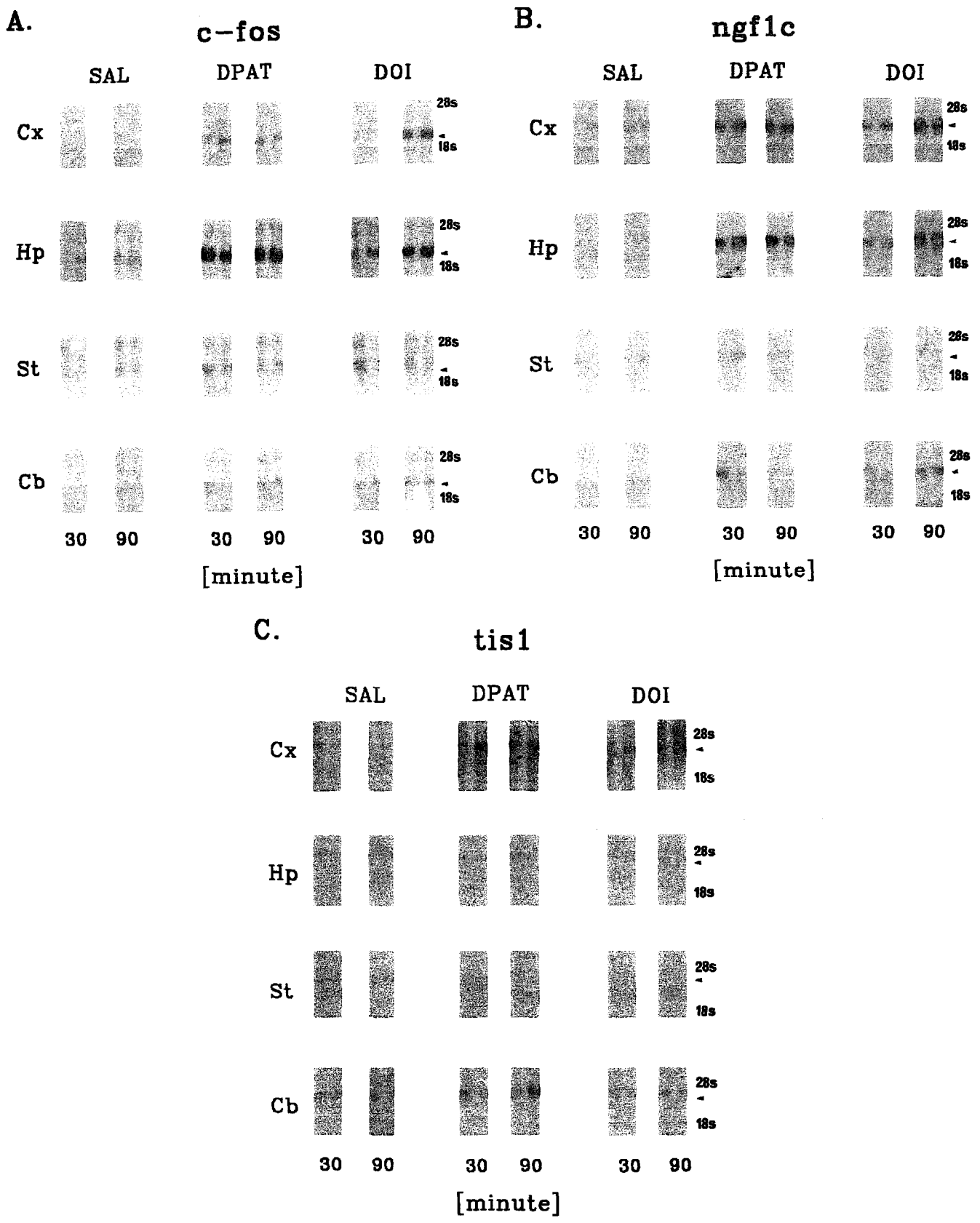

Fig. 1. Representative Northern blots to illustrate the effects of injection of SAL (saline, 0.3 ml i.p.), DPAT (8-OH-DPAT, 4 mg/kg i.p.) or DOI (DOI, 4 mg/kg i.p.) on the expression of A. *c-fos*, B. *ngf1c* and C. *tis1* mRNA in rat frontal cortex (Cx) hippocampus, (Hp), Striatum (St), and cerebellum (Cb), at 30 or 90 min post-injection. Northern blot hybridization of total RNA (10 μg per lane) from two individual rats, probed with the respective ^{32}P-labeled cDNAs, are presented. The positions of the 18S (2.0 kb) and 28S (4.8 kb) ribosomal RNA are indicated. Arrows indicate the positions of the respective immediate-early gene mRNA's.

4. Discussion

The results of this study indicate that activation of the 5-HT_{1A} or the 5-$HT_{2A/2C}$ receptor subtypes elicits substantial immediate-early gene responses in rat brain. Previously, Leslie et al. (1993) have demonstrated similar results with 5-$HT_{2A/C}$ receptor activation. An increase in *fos*-like immunoreactivity in rat brain has also been shown to be triggered by the 5-HT releasing agent, fenfluramine (Richard et al., 1992). However, the present report is the first to demonstrate immediate early gene induction by selective activation of 5-HT_{1A} receptors. Interestingly, the peak response to 5-HT_{1A} receptor stimulation occurred earlier, when compared with that resulting from 5-HT_2 receptor activation. The reason for the differential lag-time

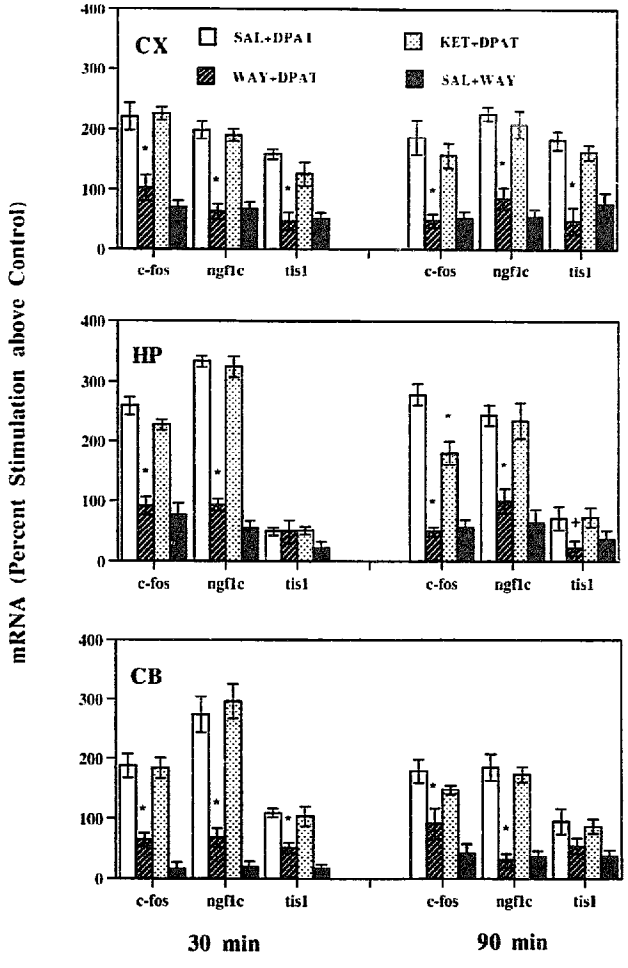

Fig. 2. Stimulation of *c-fos*, *ngf1c* and *tis1* mRNA in frontal cortex (Cx), hippocampus (Hp), and cerebellum (Cb), at 30 or 90 min following treatment with DPAT (8-OH-DPAT, 4 mg/kg i.p.) WAY+DPAT (WAY 100135, 10 mg/kg s.c. followed 20 min later with 8-OH-DPAT, 4 mg/kg i.p.), WAY+DOI (WAY 100135, 10 mg/kg s.c. followed 20 min later with DOI, 4 mg/kg) or WAY (WAY 100135, 10 mg/kg s.c. followed with saline), expressed as % stimulation above saline (0.3 ml i.p.) injected rats. Each bar represents mean ± SE of data from four individual rats. * $P < 0.05$ when compared with saline +8-OH-DPAT treatment within each immediate-early gene and post-injection time point, by ANOVA followed by Dunnett's test. + $P < 0.1$ when compared with saline injected control rats, by Student's *t*-test.

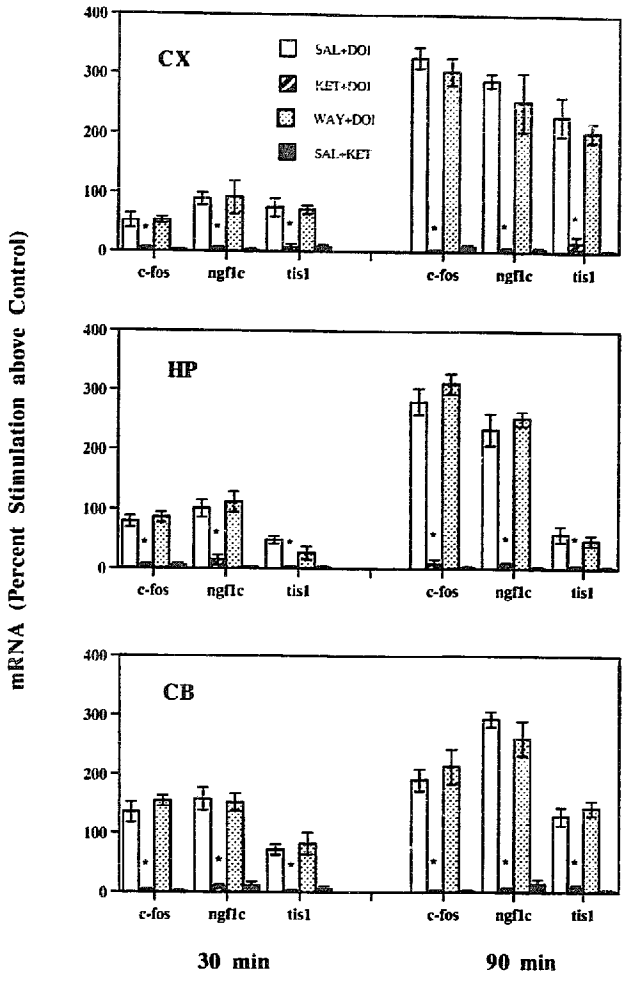

Fig. 3. Stimulation of *c-fos*, *ngf1c* and *tis1* mRNA in frontal cortex (Cx), hippocampus (Hp) and cerebellum (Cb), at 30 or 90 min following treatment with DOI (saline followed 20 min later with DOI; 4 mg/kg i.p.), KET+DOI (Ketanserin; 4 mg/kg i.p. followed 20, minutes later with DOI; 4 mg/kg i.p.), WAY+DOI (WAY 100135; 10 mg/kg s.c. followed 20, min later with DOI) or KET (ketanserin, 4 mg/kg i.p. followed 20 min later with saline), expressed as percent stimulation above saline (0.3 ml i.p.) injected rats. Each bar represents mean ± SE of data from four individual rats. * $P < 0.05$ when compared with 8-OH-DPAT injected rats, within each immediate-early gene and post-injection time point, by ANOVA followed by Dunnett's test.

is presently not clear, but may be related to differences in the signal transduction mechanisms associated with each receptor subtype (Hoyer et al., 1994). Alternatively, this differential lag-time may be a function of pharmacokinetic factors. In any event, it parallels the difference that is apparent in the behavioral responses to 5-HT_{1A} and 5-HT_2 receptor stimulation. For example, 8-OH-DPAT induced locomotor responses in rats are seen as early as 10 min post-treatment (Lund et al., 1992), whereas the classical 5-HT_2 receptor mediated responses described as the 'wet dog shake' and head twitch, on the other hand, are slower in onset (Green and Backus, 1990).

The immediate early gene responses to 8-OH-DPAT were most prominent in the frontal cortex and in the hippocampus, except for *tis1* in hippocampus. In the cere-

bellum, on the other hand, the *c-fos* and *tis1* responses were relatively weaker, although the *ngf1c* response was more robust. None of the three genes was induced by either 8-OH-DPAT or DOI in the striatum. 8-OH-DPAT is a highly selective, high-affinity 5-HT_{1A} receptor agonist (Hamon et al., 1990), and has been shown to elicit many functional responses attributed to 5-HT_{1A} receptor activation. The selective 5-HT_{1A} receptor mediated genomic response is further supported by its suppression by the phenyl piperazine derivative, WAY 100135, but not by ketanserin. WAY 100135 was shown to have high affinity for both somatodendritic and postsynaptic 5-HT_{1A} receptors (Routledge et al., 1993). Therefore, the present response pattern to 8-OH-DPAT appears to reflect the regional distribution of 5-HT_{1A} receptors. Receptor autoradiography supported by in situ hybridization to localize 5-HT_{1A} mRNA (Palacios et al., 1990) has indeed identified the hippocampus and cortex to be two regions rich in 5-HT_{1A} receptors. However, these studies have failed to demonstrate the presence of 5-HT_{1A} receptors in the cerebellum. Therefore, the present results which demonstrate 8-OH-DPAT-induced genomic responses which are sensitive to WAY 100135 together with the evidence for the presence of 5-HT_{1A} receptor mRNA in the adult rat cerebellum suggest that a 5-HT_{1A} receptor is present in the cerebellum. Previous work has identified the 5-HT_{1A} receptor in the immature cerebellum by receptor binding, immunocytochemistry and mRNA detection approaches (Miquel et al., 1994; Matthiessen et al., 1992; Matthiessen et al., 1993). The receptor appears, however, not to be easily detectable by ligand binding in the adult cerebellum. The genomic response to 8-OH-DPAT, may, however, in part, also be mediated via the somatodendritic 5-HT_{1A} receptors, the activation of which may lead to a cerebellar response through a different neurotransmitter pathway. The ability to detect direct or indirect functional responses that are not amenable to receptor binding analyses, indeed, is one of the advantages of the use of the genomic response approach in receptor studies.

Previous neurochemical, pharmacological and behavioral studies of 5-HT_{1A} receptor function have provided evidence for the selectivity of WAY 100135 as an antagonist of this receptor subtype (Fletcher et al., 1993). Furthermore, these studies have shown that this compound, compared with other 5-HT_{1A} receptor antagonists, is devoid of significant agonist activity. However, our results demonstrate a small but significant genomic response to an acute injection of WAY 100135. This genomic response may be a testament to the sensitivity of assessing immediate early response genes. Future studies with the silent 5-HT_{1A} antagonist, WAY 100635 will help resolve this issue (Forster et al., 1995). Alternatively, the response may be brought about by the blockade of the inhibitory somatodendritic autoreceptors resulting in increased 5-HT release from nerve terminals leading to the activation of post synaptic 5-HT receptors. Enhanced 5-HT release following WAY 100135 treatment has been shown previously (Starkey and Skingle, 1993). It is also possible that WAY 100135 may interact with other sites which influence immediate early gene induction. Nonetheless, the specificity of its antagonistic action on the 5-HT_{1A} receptor is supported by its lack of effect on the response to DOI.

The induction of immediate early gene expression by DOI in the cortex, hippocampus and cerebellum was completely abolished by ketanserin, indicating that it is a 5-HT_2 receptor mediated effect. The regional distribution of the response agrees with the known distribution of 5-HT_2 receptors based on ligand binding and in situ hybridization studies (Palacios et al., 1990), as well as by receptor-mediated *c-fos* gene induction (Leslie et al., 1993).

Both short and long-term consequences of transmembrane signalling are evidently mediated by changes in gene expression. Some genes are activated within minutes of stimulation, while others are induced, or are repressed more slowly, via a mechanism dependent on new protein synthesis (Sheng and Greenberg, 1990). Analysis of receptor mediated gene expression has also indicated possible differences in the types of genes which are either stimulated or repressed depending on the nature of the stimulus. Iwaki et al. (1990) found that the stimulation of either α_1- or β-adrenoceptors could activate the *c-fos* gene in cardiac myocytes, whereas *zif268* gene is activated by the α_1 receptor only. Present results also suggest a possible trend of differential immediate early gene response. In the cortex, all three genes studied are induced by either 5-HT_{1A} or 5-$HT_{2A/C}$ receptor activation. However, in the hippocampus, only *c-fos* and *ngf1c* are induced, but not *tis1*, by either of the receptor subtypes. In the cerebellum, both 5-HT_{1A} and 5-$HT_{2A/C}$ receptor activation causes the induction of all three genes. Neither 8-OH-DPAT nor DOI is capable of inducing the expression of any of the three genes in the striatum. Previous studies have demonstrated the capacity of the rat hippocampus to respond to different stimuli with much greater *c-fos* response than that observed by us with 5-HT-receptor activation (Bading et al., 1993; Massieu et al., 1992). This indicates that the rat hippocampus does possess the adequate transcriptional capacity for higher levels of *c-fos* induction. As such, the relatively lower response observed in the present studies may reflect differences which are related to the nature of the stimulus, or the dose of the agonist. It will be of interest to examine this aspect of receptor mediated gene expression further to determine the possible existence of a stimulus-specific and a predictable pattern of differential gene activation. The latter, would support the idea that, although immediate early gene induction is a response common to a wide variety of stimuli, the specificity of cellular response to a given stimulus may be the result of the activation of a specific set of genes (Dragunow et al., 1989).

In conclusion, the activation of the 5-HT_{1A} or the 5-HT_2 serotonin receptor subtypes induces the expression of im-

mediate early genes. Based on this criterion, the activities of both of these receptor subtypes are localized to the cortex, hippocampus and cerebellum of rat brain. The present results also suggest a spatial and temporal pattern of immediate early gene expression in response to central 5-HT_{1A} and 5-HT_2 receptor activation. In view of the failure to demonstrate of 8-OH-DPAT binding in the adult cerebellum in previous studies, the finding of a genomic response to the drug, the demonstration of 5-HT_{1A} receptor mRNA in this brain region and previously demonstrated functional effects of 5-HT_{1A} receptor stimulation (Maura and Raiteri, 1996) clearly attest to the significance of the serotonergic system in this brain region.

Acknowledgements

The gift of WAY 100135 by Wyeth Research (U.K.) is gratefully acknowledged. This work was supported by a grant from the Medical College of Pennsylvania's Intramural Research Pilot Program and by USPHS grants #AG07700, #DA06871, #MH45166. The help of Vanessa Cain in preparing the manuscript is gratefully acknowledged.

References

Bading, H., D. Ginty, and M.E. Greenberg, (1993) Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways, Science 260, 181–186.

Chomczynski, P. and N. Sacchi, (1987) Single step method of RNA isolation by acid guanidium thiocyanate phenol chloroform extraction. Anal. Biochem. 162, 156–159.

Davis, L.G., M.D. Dibner, and J.F. Battey, (1986) Basic Methods in Molecular Biology Elsevier, N.Y., pp. 147–149.

Dragunow, M., R.W. Currie, R.L.M. Faull, H.A. Robertson, and K. Jansen, (1989) Immediate early genes, kindling and long-term potentiation. Neurosci. and Biobehav. Rev. 13, 301–313.

Fletcher, A., I.A. Cliffe, and C.T. Dourish, (1993) Silent 5-HT_{1a} receptor antagonists: utility as research tools and therapeutic agents, Trends Pharmacol. Sci. 14, 441–448.

Forster, E.A., I.A. Cliffe, D.J. Bill, G.M. Dover, D. Jones, Y. Reilly and A. Fletcher, (1995) A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635, Eur. J. Pharmacol. 281, 81–88.

Green, A.R. and L.I. Backus, (1990) Animal models of serotonin behavior. In P.M. Whitaker- Azmitia and S.J. Peroutka (Eds.), The Neuropharmacology of Serotonin, Vol. 600, Ann. N.Y. Acad. Sci., New York, pp. 237–248.

Hamon, M., H. Gozlan, S. El Mestikawy, M.B. Emerit, F. Bolanos, and L. Schechter, (1990) The central 5-HT_{1a} receptor: Pharmacological, biochemical, functional and regulatory properties. In P.M. Whitaker-Azmitia and S.J. Peroutka (Eds.), The Neuropharmacology of Serotonin, Vol. 600, Ann. N.Y. Acad. Sci., New York, pp. 114–131.

Hoyer, D., D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena and P.P.A. Humphrey, (1994) International Union for Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–203.

Iwaki, K., V.P. Sukhatme, H.E. Shubeita, and K.R. Chien, (1990) Alpha and beta adrenergic stimulation induces distinct patterns of immediate early gene expression in neonatal myacaridal cells. J. Biol. Chem. 265, 13809–13817.

Leslie, R.A., J.M. Moorman, A. Coulson and D.B. Graham-Smith, (1993) Serotonin$_{2/1C}$ receptor activiation causes a localized expression of the immediate-early gene c-*fos* in rat brain: evidence for involvement of dorsal raphe nucleus projection fibers, Neurosci. 53:(2), 458–463.

Lim, R., B. Varnum, and H. Herschman, (1987) Cloning of tetradecanoyl phorbol ester- induced primary response' sequences and the expression in density-arrested Swiss 3T3 cells and a non-proliferative variant, Oncogene 1, 263–270.

Lund, A., N. Mjellum-Joly, and K. Hole, (1992) Desipramine, administered chronically, influences 5-hydroxytryptamine$_{1A}$-receptors, as measured by behavioral tests and receptor binding in rats, Neuropharmacol. 31, 25–32.

Massieu, L., R. Rocamona, J.M. Palacio, and W.G.M.B. Hendrikus, (1992) Administration of quinilonic acid in the rat hippocampus induces expression of c-fos mRNA, Mol.Brain Res. 16, 88–96.

Matthiessen, L., G. Daval, Y. Bailly, H. Gozlan, M. Hamon and D. Verges, (1992) Quanitification of 5-hydroxytryptamine$_{1A}$ receptors in the cerebellum of normal and x-irradiated rats during postnatal development, Neurosci. 51 (2), 475–485.

Matthiessen, L., H. Kia, G. Daval, M. Riad, M. Hamon and D. Verge, (1993) Immunocytochemical localization of 5-HT_{1A} receptors in the rat immature cerebellum, NeuroReport 4, 763–766.

Maura, G. and M. Raiteri, (1996) Serotonin 5-HT_{1D} and 5-HT_{1A} receptors respectively mediate inhibition of glutamate release and inhibition of cyclic GMP production in rat cerebellum in vitro, J. Neurochem. 66:(1),203–209.

Miquel, M., H. Kia, C. Boni, E. Doucet, G. Daval, L. Matthiessen, M. Hamon and D. Verge, (1994) Postnatal development and localization of 5-HT_{1A} receptor mRNA in rat forebrain and cerebullum, Develop. Brain Res. 80, 149–157.

Morgan, J.I. and T. Curran, (1991) Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes, fos and jun. Ann. Rev. Neurosci. 14, 421– 451.

Palacios, J.M., C. Waeber, D. Hoyer, and G. Mengod, (1990) Distribution of Serotonin receptors, Annals N.Y. Acad. Sci. 600, 36–50.

Richard, D., S. Rivest, and C. Rivier, (1992) The 5 hydroxytryptamine agonist fenfluramine increases fos like immunoreactivity in the brain. Brain Res. 594, 131–137.

Routledge, C., J. Gurling, I.K. Wright, and C.I. Dourish, (1993) Neurochemical profile of the selective and silent 5-HT_{1a} receptor antagonist WAY 100135: an in vivo microdialysis study, Eur. J. Pharmacol. 239, 195–202.

Sagar, S.M., F.R. Sharp, and T. Curran, (1988) Expression of c-fos protein in brain: metabolic mapping at the cellular level, Science 240, 1328–1331.

Sheng, M. and M.E. Greenberg, (1990) The regulation and function of c-fos and other immediate early genes in the nervous system, Neuron 4, 478–485.

Starkey, S.J. and M. Skingle, (1993) Effects of WAY 100135, 8-OH-DPAT, and sumatriptan on 5-HT release evoked by a single electrical pulse in slices of guinea pig dorsal raphe nucleus, Br. J. Pharmacol. 108, 100P.