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Isolation and Characterization of the Rat 5-Hydroxytryptamine Type 2 Receptor Promoter: Constitutive and Inducible Activity in Myometrial Smooth Muscle Cells

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SUMMARY

Previous studies from this laboratory have demonstrated that the 5-hydroxytryptamine (5-HT_2) receptor subtype is transcriptionally regulated by 5-HT (serotonin) itself in rat myometrial smooth muscle cells. To better understand this transcriptional regulation, we have isolated and characterized the 5′-flanking region of the 5-HT_2 receptor gene. Screening of a rat genomic library was accomplished using 5′-directed fragments of 5-HT_2 cDNA, and a 5.2-kilobase fragment was isolated. Sequencing demonstrated that the fragment overlapped the 5′-end of the 5-HT_2 cDNA by 226 base pairs. Primer extension and RNase protection analyses indicated that three transcriptional start sites, which are common to both rat brain and myometrium, appear to exist and that the 5′-untranslated region of the 5-HT_2 receptor cDNA is 1120 base pairs long. Neither classical TATA boxes nor CCAAT sequences were found upstream of any of the start sites identified. Upstream of the dominant start site, however, an initiator consensus sequence, two GC boxes (SP-1 binding sites), and several AP-2 binding sites were identified. Based on this information, a 1.4-kilobase fragment beginning 64 base pairs downstream from the dominant start site was constructed by polymerase chain reaction and ligated into a pCAT vector. Transient transfection of this construct into rat myometrial smooth muscle cells displayed both constitutive and serotonin-induced promoter activity. Serotonin-inducible activity was abolished by a selective 5-HT_2 receptor antagonist; however, antagonists selective for other 5-HT receptor subtypes were without effect. Conversely, a selective 5-HT_2 receptor agonist completely substituted for serotonin as an inducer. Preliminary deletion experiments indicate that regulation of basal and serotonin-inducible activity likely depends upon different *cis* elements in the 5-HT_2 receptor gene promoter.

The 5-HT_2 receptor subtype has been identified in numerous tissues, including brain, several smooth muscle-rich organs, and platelets. Its modulation has been related to a variety of pathological neurological states (1), and its native agonist, serotonin, has been shown to stimulate the contraction of smooth muscle in a number of peripheral tissues. Regulation of central 5-HT_2 receptors does not result in the traditional adaptive pattern of brain monoamine receptors, wherein agonist treatment typically causes down-regulation of receptor number and antagonist treatment results in receptor up-regulation. Rather, both antagonists and agonists down-regulate central 5-HT_2 receptors (2, 3). These studies suggest that the 5-HT_2 receptor may have unique regulatory mechanisms. Recent studies from this laboratory have shown that cultured rat myometrial smooth muscle cells express 5-HT_2 receptors, possibly as the only 5-HT receptor subtype present, and that serotonin activates the gene for interstitial collagenase in these cells via interaction with this receptor (4, 5). Of particular interest is our finding that serotonin also activates the gene for the 5-HT_2 receptor in these cells (6). This activation is mediated by the 5-HT_2 receptor itself; selective 5-HT_2 agonists fully mimic serotonin, and antagonists prevent the up-regulation.

To elucidate the mechanism of this unusual regulation of the 5-HT_2 gene, we sought to isolate the promoter region of the gene and examine it, in transient transfection experiments, for elements that are important in regulation. The present report presents the first results of this effort. We have isolated and sequenced 1.4 kb of the 5′-flanking region of the 5-HT_2 promoter region as well as the remaining, heretofore unreported, portion of the 5′-UTR of the mRNA. The 5-HT_2 receptor gene promoter is TATA-less but contains a sequence very similar to the Inr element identified in the promoter regions of a variety of genes without canonical TATA boxes (7). In transient transfection experiments we demonstrate that the transcriptional

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; kb, kilobase(s); bp, base pair(s); UTR, untranslated region; CAT, chloramphenicol acetyltransferase; PCR, polymerase chain reaction; Inr, initiator; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl.

ability of the promoter is in part constitutive and in part regulated by 5-HT, via the 5-HT_2 receptor, in myometrial smooth muscle cells. These results suggest the existence of a complex set of regulatory mechanisms, whereby constitutive and inductive activation are regulated independently. Lastly, we have identified an open reading frame in the 5′-UTR, beginning with a classical start codon and terminated by a stop codon, that codes for a putative protein of 64 amino acids in length. This finding suggests the possibility of translational control mediated by this open reading frame in the 5′-UTR of the 5-HT_2 receptor mRNA.

Materials and Methods

Isolation of the 5′-flanking region of the 5-HT_2 receptor gene. Genomic screening was performed by standard methodologies (8), using a commercially available genomic library from rat testis (Stratagene, La Jolla, CA) and using a cDNA for the rat 5-HT_2 receptor as a probe (a kind gift from Dr. Beth J. Hoffmann, National Institute of Mental Health, Bethesda, MD). Approximately 6×10^5 plaques were screened. Hybridization and wash conditions were as described previously (9). Filters (Colony/Plaque Screen; DuPont/NEN, Wilmington, DE) were first hybridized with the full length cDNA. Candidate clones were then rescreened with a 5′-fragment derived from the full length cDNA by *Bam*HI/*Sac*II digestion (see Fig. 1). DNA was purified, ligated into pBluescript SK$^-$ (Stratagene), and sequenced by standard dideoxynucleotide chain termination methodology (Sequenase, version 2.0; United States Biochemicals, Cleveland, OH). DNA homology searches were performed using DNASIS software (Hitachi Software Ltd., San Bruno, CA).

RNA preparation. Total RNA from tissues or cultured cells was prepared with minor modifications of previously published procedures (10). Briefly, cells or tissues were lysed in 4 M guanidine isothiocyanate, 0.5% *N*-laurylsarcosine, 25 mM sodium citrate, pH 7.0, 0.7% β-mercaptoethanol, using a Dounce or motor-driven homogenizer. Cell lysates were layered onto 96% CsCl, 25 mM sodium acetate, pH 5.2, and RNA was pelleted for 22 hr at 174,000 × *g*. RNA pellets were dissolved in distilled water, precipitated with 0.3 M sodium acetate/70% ethanol, washed, and resuspended in distilled water before use.

Primer extension and RNase protection analysis. For primer extension, an oligonucleotide (Fig. 1) corresponding to the 5′-most sequence of the 5-HT_2 receptor cDNA (5′-CCCTCTCTTTAAGCTTC CAGCAGCATAAGA-3′) was 5′-end labeled with [γ-^{32}P]ATP, using polynucleotide kinase, and was purified on NENSORB (DuPont/NEN). Total RNA (50 μg from rat frontal cortex or 15 μg from uterine smooth muscle cells) was hybridized to the aforementioned primer, and extension was accomplished by the addition of Moloney murine leukemia virus reverse transcriptase (GIBCO-BRL, Grand Island, NY), as described previously (8). For RNase protection assays, a 1.5-kb [α-^{32}P]UTP-labeled antisense RNA starting from oligonucleotide 1 (Fig. 1) was synthesized with T7 RNA polymerase, using a commercial kit (MAXIscript; Ambion Inc., Austin, TX). After overnight hybridization with total RNA from the appropriate source, single-stranded RNA was digested with RNases T1 and A (8). Yeast RNA (20 μg) was used in place of cellular RNA as a negative control. The products of primer extension and RNase protection assays were analyzed on 5% polyacrylamide gels containing 8 M urea (8). DNA sequence ladders were used as molecular weight markers.

Cell culture and transient transfection of rat myometrial smooth muscle cells. Rat myometrial cells from 4-day postpartum uteri were prepared as described in detail previously (11). Cells actively producing collagenase were equilibrated to serotonin-free conditions by incubation for 3 days in medium-containing serum that had been adsorbed with dextran-coated charcoal (4). Cells were provided with fresh serotonin-free medium 30 min before transfection. Cells were transfected by the calcium phosphate precipitation method (12) for 5 hr in all experiments. Approximately 5.6 pmol (20 μg for largest

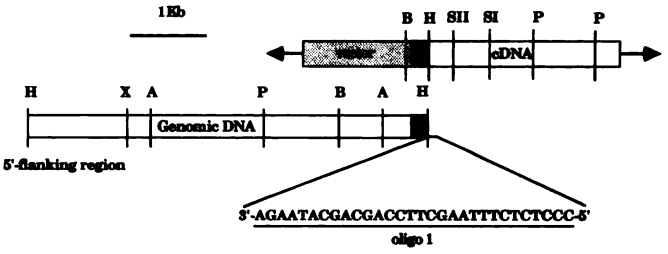

Fig. 1. Restriction map of the 5-HT_2 receptor cDNA, 5′-UTR and the 5′-flanking region cloned in this study. The region of the cloned fragment overlapping with the 5-HT_2 cDNA is in *black*. The primer used in the primer extension (oligonucleotide 1) is *underlined*. Restriction enzyme sites are identified as follows: *A*, *Acc*I; *B*, *Bam*HI; *H*, *Hind*III; *P*, *Pst*I; *SI*, *Sac*I; *SII*, *Sac*II; *X*, *Xba*I.

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                                  -1314
5'- CATTCATGCA TGTTATCTTA TACCTCACTG TAACCCAGGG ATGATGAAGC TAATAACGAA
    CTGGGAGTGG GGAGCAGTGG GCTCTAAAGG CAGCTGAGTT AGGTTATATG TGTTACAAAG
    CTGGATTCTG AACCAAAGGA CTGTGACTCT CACACCTACT CTGTACACAC ATGACCGCAG
    CCTAGTCACA GGTAGGCGAT CTGGGAAATA ATGTCCCTGC CCAGGAAGAG CAGAAGGTCT
    CCTCACTGTG GGCTGAGAAG AGGAGAGCTG GGCTGAGCAG AGGAGAGCTG AGAACTGTGA
    GTGGGATGGG AGCAATGCTG GGAACCTAGA GGCACAGGCA GGGAGCAGGG ATGAAGGGCC
    AGGTGGACCA TATGATGAGT CCATGAGCCC TAAGAGGAGG GTCAGGATGG ATCTCTGGGT
    CACAGACAGA AGGTCTAGAA GGCAACTAGA CATTTAGCTC ACTGCCATGA GAACACACTG
    AAGGCCTGGG AGTGGCTGAA CTGAGAGTGT AAAAGATAAA CAAGAGAAAA ATTAGGTGGA
      AP-2
    CAGAACCCCC AAAAATCCCT GATGTCCAGG AAATAATCGA TTTTCTTATC TGTCATGAGT
    ACATCTATCT TCCTTTTTTT TTTTTTTTT TTCCGGAGCT GGGGACCGAA CCCAGGGCCT
                                                          AP-2
    TGCGCTTCCT AGGCAAGCGC TCTACCACTG AGCTAAATCC CCAGCCCCTA CATCTATCTT
    CCTTTAAGGA CCACTTGGGA GAGGGCCTCT TTTTCATCTA GTCRCTTTAT CATTAGGGTC
    ACYTCTGGAA GAAATAAAAA TAGRACAAAA CCCTTCACYT TCTAACATGA AGTTTTCAAT
    TACCTNTTGG CAACCTTTTT CAGAACAGAA GAAAAGTAAG CTAGTTGCGA GATGTATACA
    CATAACAATA GTGCCTGTGC TTTAACTAAC AAAAATTGTT CTAATAGTTT TATACGTTAT
    TGCCAACGAC TTCCTTGACT CCTCTGAACG TGTGTCACCA CTGTCAGCCA TCCCCTCCTG
    GACACATAAC CACTGGTGTG GCTAGAAGTG CCAGATGCCC CTAGATCCCA GGACGTGCGG
    TTTTCTAGCC GATTATTTAT TTTCTCACAC TTCGCCCCTC AGCCTAAAAT ATGACTGCCA
    TGTAGCCACC TGCCTGCCAT CCCCTAGGCT GTCTCCCTCG ATTCTCTCTT TTTTATGGAA
                        AP-2
    AGGTTCTCAA TTAACCTGTA GCAGAAAGAA GAATGGGAGT GCCGGGAGGG GCGGTTTGGT
                  -75                                       Sp-1
    CCGCCCTTCC TCGTGTCGCC ACCTTGCAGG TTATGACACA GTGACAGAGG GAGGTCTGAG
    Sp-1                                  +1
    GAAGGAGGCA ACCAGGAGCT GACTTATTCT TCTGGAAATC CCAGAGCCTC CTGGTACAGA
                          Inr                +64
    CAGCGTTTCC TACTTTCCTG CGGCTCTTTT GTGTGACTTT GAGAGGGGCT CTCTGGTGAC
    TGATTCCTCT CTGTGCGCTC GCCTCAGCGA CGAGCCAGGC TCTGGGAGGG AGGACCCAAG
    CTCAGCAGTG GCAGAGGCAG CCAGCACAGA CCTCAACGGT GCTGGAACCG GCATGCGGTT
    TTTGAAGTCA GCAAAACCAG AAATCAAATT ACTATCATCT TATGCTGCTG GAAGCTT -3'
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Fig. 2. Sequence of the 5′-flanking region of the 5-HT_2 receptor gene. *Arrows*, the three transcriptional start sites identified by both primer extension and RNase protection. Consensus sequences are *underlined*. *, The end of the rat 5-HT_2 cDNA that was used as a probe for screening. Bases −1314 and −75 are at the 5′-ends of the constructs used in transfection/CAT assays.

construct) of pCAT plasmid (Promega, Madison, WI) containing the appropriate construct were utilized for each transfected 75-cm^2 flask. After transfection, cells were washed with serotonin-free medium, incubated for 20 min with 5-HT_2 antagonists as appropriate, and then incubated in medium containing 5 μM 5-HT and supplemented with agonists or antagonists as appropriate to the experiment. After 48 hr, cell extracts were prepared by washing the cells twice with phosphate-buffered saline (12) and scraping the cell layer from the flask, with a rubber policeman, into 300 μl of 0.1 M Tris·HCl, pH 7.8. Cells were lysed by three cycles of freeze-thawing and incubated for 10 min at 65° to inactivate endogenous acetylases, and cell debris was removed by

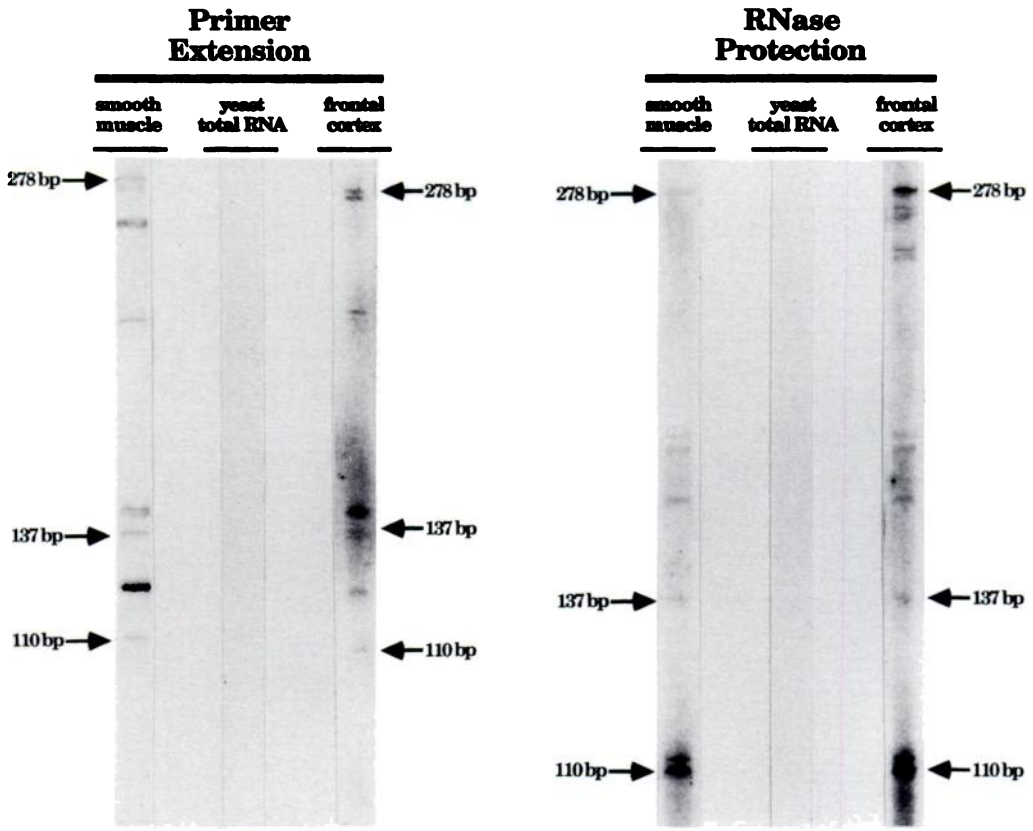

Fig. 3. Primer extension and RNase protection analysis to determine the transcriptional start sites of the 5-HT$_2$ mRNA. *Arrows*, sites found to be common to myometrial smooth muscle and the frontal cortex of brain by both methods. Sizes indicated are in base pairs from the 5′-end of oligonucleotide 1 (see Fig. 1). Photograph shown is from one of three experiments. Bands indicated are products that were present in all three experiments.

centrifugation at 16,000 × *g*. Protein concentrations were measured according to the method of Bradford (13), using a commercial kit (Bio-Rad, Richmond, CA). CAT enzyme activity in 20 μg of cell extract protein was determined in the presence of [^{3}H]acetyl-coenzyme A (DuPont/NEN), according to the diffusion method of Neumann *et al.* (14). CAT activity was determined in a liquid scintillation counter for at least three time points. In all experiments the reaction was linear for at least 6 hr. Typically, activity in basally induced cell extracts was 1000–2000 cpm/hr. The value obtained from the transfection of the promoterless basic pCAT vector was assigned a value of 1 unit of CAT activity. Experiments were repeated three or four times (with different batches of primary cultures), in each of duplicate 75-cm^2 flasks.

Assessments of possible variations in transfection efficiency were made by co-transfecting cells with the positive control vector pSV-β-Gal (Promega) for the same times as for the experimental plasmids. The cellular content of β-galactosidase was then measured by enzymatic assay, using a commercial kit (Promega).

Recombinant PCR. Recombinant PCR was used exactly as described previously (15), to delete the putative Inr sequence (TATTCTTCT) present from −9 to −1 bp in the 5′-flanking region of the 5-HT$_2$ promoter. After ligation into the pCAT vector, the deletion was verified by sequencing.

Results

Isolation of the 5′-flanking region of the 5-HT$_2$ receptor gene. Screening of the rat genomic library with a full length 5-HT$_2$ receptor cDNA yielded 72 positive clones. Subsequent screening with a *Bam*HI/*Sac*II fragment of the cDNA, corresponding to the 5′-most region of the cDNA, resulted in the plaque purification of two clones. One, a 5.2-kb *Hind*III fragment, hybridized with the *Bam*HI/*Sac*II fragment but with none of the more 3′-oriented fragments derived from the cDNA. This fragment, therefore, was most likely to contain the promoter region of the 5-HT$_2$ receptor. The fragment was subcloned into pBluescript SK$^-$, and a restriction map was generated (Fig. 1). A partial sequence of the fragment, using a primer based on sequences at the 5′-end of the cDNA, revealed that the cloned fragment overlapped by 226 bp with the 5′-end of the cDNA and contained consensus sequences characteristic of a promoter region (Fig. 2). Although no TATA box was identified, an Inr sequence (TTATTCTTC), similar to that found in a number of TATA-less promoters, was found, as were SP-1 and AP-2 binding sites. The two SP-1 sites are 80 bp upstream from the Inr sequence. To establish this region as that of the promoter, it was necessary to first identify the transcriptional start site(s) of the 5-HT$_2$ gene.

Identification of the 5′ boundary of the 5-HT$_2$ receptor cDNA. To determine the transcriptional start site of the 5-HT$_2$ receptor gene, primer extension analysis was performed using oligonucleotide 1 (Fig. 1). Rat frontal cortex RNA was also included in the experiment, to produce analogous information from a tissue that is rich in the 5-HT$_2$ receptor. The data from both analyses indicated the existence of three transcriptional start sites, identical in the RNA from both tissues, at 110 bp, 137 bp, and 278 bp from the end of oligonucleotide 1 (Fig. 3). The band at 278 bp not only was present in both tissues but also was located immediately downstream from the only initiator sequence identified in the promoter region. Thus, this locus was provisionally designated as the predominant

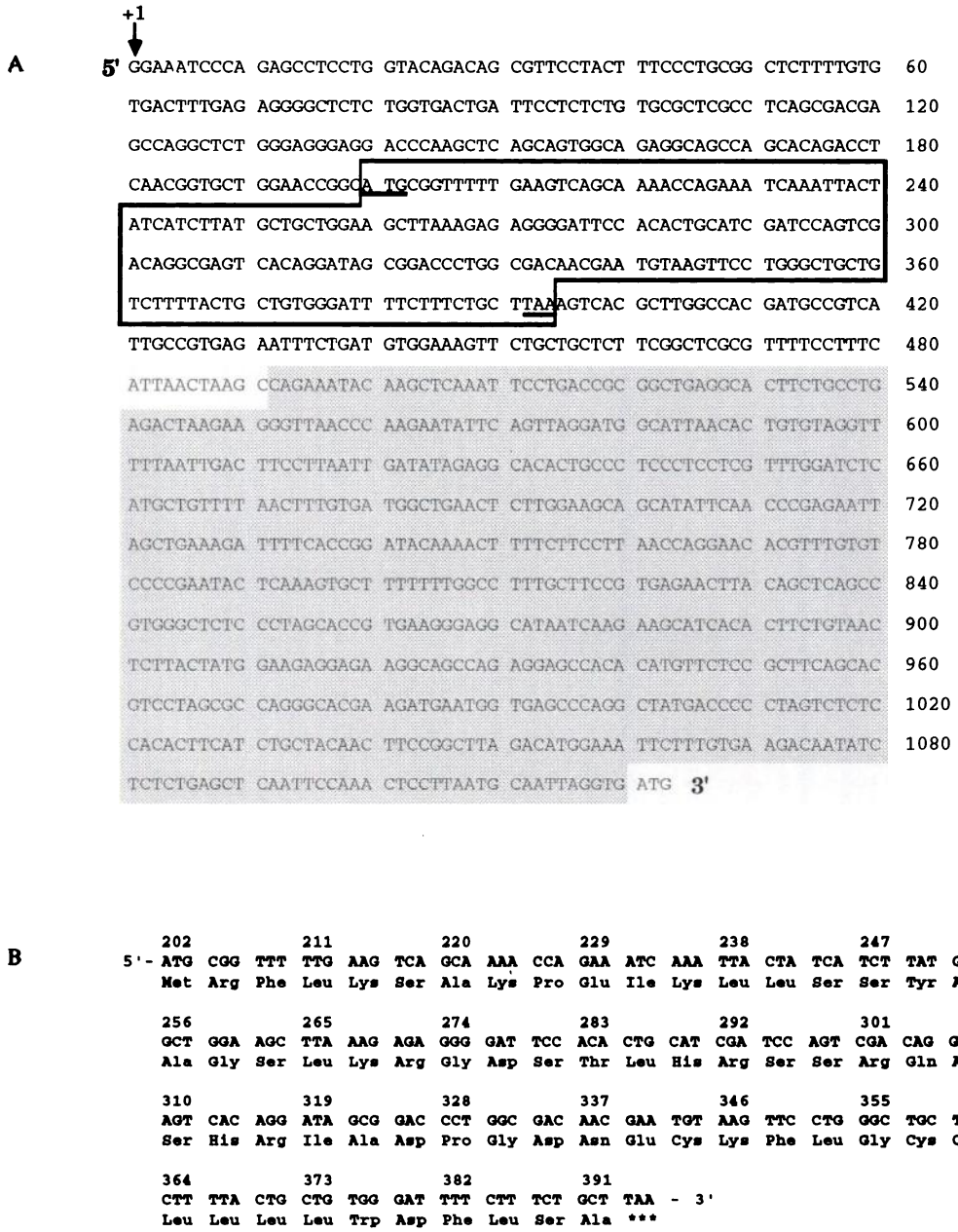

Fig. 4. Sequence of the 5-HT_2 receptor cDNA 5′-UTR. A, The region of overlap between the previously published sequence (17) and that presented here is *shaded*. The ATG codon beginning at position +1121 is the start site of translation of the 5-HT_2 receptor. The *boxed region* from position +200 to position +394 is the longest open reading frame in the 5′-UTR. B, Nucleotide sequence and amino acid sequence of the putative protein encoded in the rat 5-HT_2 5′-UTR.

transcriptional start site of the 5-HT_2 receptor. Controls in which yeast RNA was substituted for cellular RNA revealed no bands on the gel (Fig. 3), suggesting that the bands observed when either brain or smooth muscle RNA was used represent transcriptional start sites.

Sequence and characterization of the 5-HT_2 receptor cDNA 5′-UTR. The cloning of the cDNA for the rat 5-HT_2 receptor has been reported by several groups (16, 17); none of the sequences published to date, however, has provided the sequence of the entire 5′-UTR. The cDNA with which we began this study was longer, by 453 bp, than the longest sequence previously published (Fig. 4A). The sequence obtained in the present study identifies yet an additional 38 bp of 5′-untranslated sequence, for a total of 1120 bp, counting from the predominant transcriptional start site as shown in Figs. 2 and 4. The homology between the sequence of our cDNA and regions of previously published cDNAs (16, 17) is shown in Fig. 4A and is approximately 98%. The 5′-UTR of the rat 5-HT_2 receptor cDNA contains a number of ATG codons, three of which are at the beginning of open reading frames. The longest of these, extending from position +200 to position +394, encodes a putative protein of 64 amino acids in length (Fig. 4B).

Constitutive expression and serotonin induction of the 5-HT_2 receptor in transient transfection studies. The functional ability of the 5-HT_2 promoter was assessed by transient transfection assays. A 1.4-kb fragment of the 5.2-kb genomic clone (positions −1314 to +64 with respect to the predominant transcriptional start site) was ligated upstream of

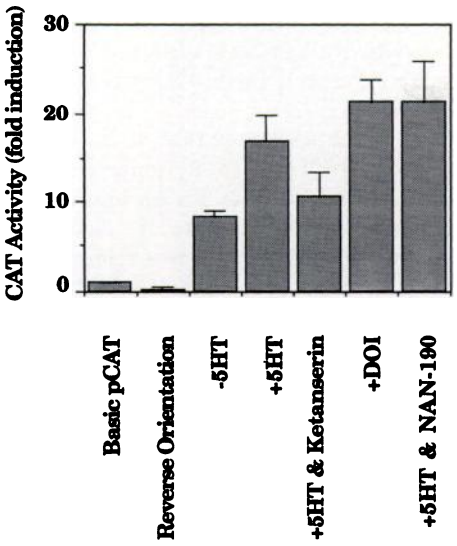

Fig. 5. 5-HT$_2$ receptor-mediated induction of transcription. Data were standardized by assigning the constitutive activity of the promoterless pCAT vector a value of 1. After transfection, agonists (5-HT and DOI) were added to serotonin-free medium for 48 hr. When antagonists were used, the cells were pretreated for 20 min with appropriate antagonist and the cells were then cultured for 48 hr in medium containing 5-HT and antagonists. Cell extracts were prepared as described in Materials and Methods, and 20 μg of protein were used in each CAT assay. Each value represents the mean ± standard error of three or four independent experiments, each of which was performed with duplicate flasks for each treatment.

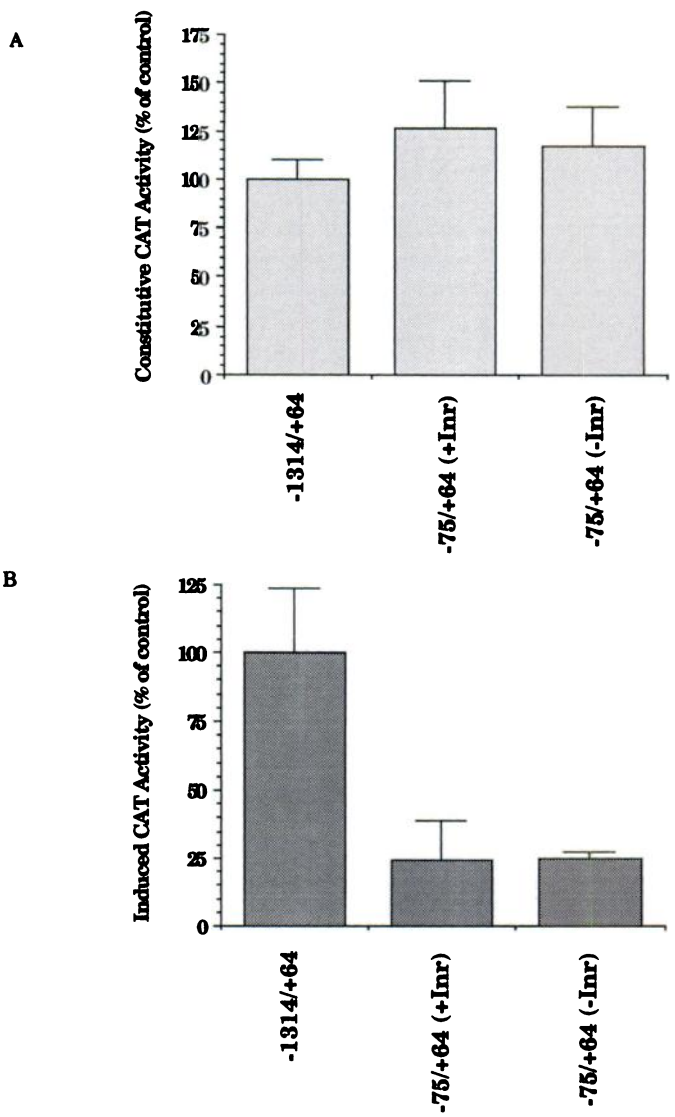

Fig. 6. Effect of deletion of the Inr element on constitutive and inducible 5-HT$_2$ promoter activity. A, Constitutive activity of −75/+64(+Inr) and −75/+64(−Inr) constructs. The constitutive activity of the full length (−1314 to +64) promoter construct was assigned a value of 100%. B, Inducibility of promoter activity by DOI (5 μM). The inductive activity of the full length (−1314 to +64) promoter construct was assigned a value of 100%. All values are the mean ± standard error of four independent experiments.

the CAT gene and verified for correct orientation. After transfection, the constitutive CAT activity was approximately 8-fold higher than that displayed by either the promoterless pCAT or pCAT into which the 1.4-kb fragment had been ligated in the reverse orientation (Fig. 5). Treatment of the cells with 5 μM serotonin further induced CAT transcription by approximately 3-fold. Serotonin failed to increase the activity of either the pCAT- or reverse orientation promoter-transfected cells (data not shown). The effect of serotonin was completely mimicked by 5 μM DOI, a specific 5-HT$_2$ receptor agonist. Conversely, the selective 5-HT$_2$ antagonist ketanserin (200 nM) blocked the induction by serotonin. A selective antagonist of the 5-HT$_{1a}$ receptor, NAN-190, failed to prevent the induction by serotonin (Fig. 5). Similarly, the selective 5-HT$_3$ antagonist ICS-205193 had no effect on serotonin-induced promoter activity (data not shown). Co-transfection of the cells with the constitutively expressed control plasmid pSV-β-Gal was routinely performed to assess any differences in transfection efficiencies. In all cases, the expression of β-galactosidase in extracts of cells transfected with a variety of plasmids and incubated either in the presence or in the absence of serotonin displayed differences of <10% between flasks in any experiment (data not shown).

In preliminary experiments designed to examine the role of the Inr sequence in promoter activity, recombinant PCR was used to synthesize constructs representing the sequence of the promoter region from base −75 to base +64, either containing the intact Inr element or with the Inr deleted. The results (Fig. 6) show that the constitutive activity of the promoter remained intact in the absence of Inr. In addition, neither construct displayed DOI-dependent induction.

Discussion

In this study we have isolated and characterized the promoter region for the 5-HT$_2$ receptor gene. The 5-HT$_2$ promoter does not contain either a classical TATA box or a CAAT box, as do most mammalian promoters, including that of the mouse 5-HT$_{1c}$ receptor (18). Rather, the 5-HT$_2$ promoter contains several AP-2 sites, two SP-1 sites, and a sequence identical to an initiator consensus sequence, designated Inr (YAYTCYYY, where Y is any pyrimidine). Underlined A and TC are invariant (19). In the 5-HT$_2$ promoter, the Inr sequence (TATTCTTC) appears at positions −1 to −9 with respect to the major transcriptional start site. The Inr sequence has been found in a number of genes lacking a TATA box and has been found to be important in directing transcription in these genes. Interestingly, a number of receptor genes seem to lack classical TATA boxes but contain the Inr sequence; interleukin-1 (20), epidermal growth factor (21), insulin-like growth factor (22), and nerve growth factor (23) receptors are among this group, as are a group of genes associated with cellular housekeeping

functions, such as hypoxanthine-guanine phosphoribosyltransferase and adenosine deaminase (for review, see Ref. 7).

Examination of the 5-HT_2 cDNA by both primer extension and RNase protection methodology suggests the existence of several transcriptional start sites, three of which are identical in myometrial smooth muscle cells and in the frontal cortex of the brain. It is not clear whether, or how, these sites are utilized for transcriptional activation. In the two rat tissues we have examined, frontal cortex and uterine smooth muscle, two mRNA transcripts are commonly observed by Northern blot analysis, differing by 400–500 bp (6). This finding confirms the original observation of Julius *et al.* (16) in the frontal cortex. Whether this finding indicates that multiple transcripts exist or that differential processing occurs is unknown for either tissue. Our data would be consistent with the utilization of alternate start sites.

Surprisingly, the nature and number of enhancer sequences and their organization in the rat 5-HT_2 receptor promoter are quite different from those in the recently reported mouse 5-HT_{1c} receptor promoter (18). The cDNAs and the protein organization of the two receptor subtypes are closely related, although the binding affinity of serotonin for the two receptors is quite different. Clearly, from inspection of their promoter regions, the regulation of the two genes is also likely to be quite different. On the other hand, the two subtypes both display 5′-UTRs that are quite long for mammalian cDNAs (1120 bp for rat 5-HT_2 receptor and 855 bp for mouse 5-HT_{1c} receptor). The reason for these very long 5′-UTR regions is unknown. Evidence is accumulating from a variety of systems, however, that such long regions, particularly when they contain methionine start codons, are important in the translational regulation of the protein encoded by the mRNA (24). In the case of the 5-HT_2 5′-UTR, several such codons exist, the longest of which appears at the beginning of an open reading frame from position +200 to position +394 in the 5′-UTR. The open reading frame codes for a putative protein of approximately 6800 Da. The putative protein contains exactly 50% hydrophobic residues (32 of 64). Of the 18 charged amino acids, 12 are basic. The existence of such reading frames in the 5′-UTR of the transforming growth factor-β cDNA has been shown to be responsible for the control of the translation of the mRNA (24). It is not known whether such a role exists for this region in the 5-HT_2 receptor 5′-UTR; studies are presently in progress to investigate this possibility.

In our transient transfection studies with the 1.4-kb 5-HT_2 promoter, we find that basal promoter activity, that is, transcriptional activity in the absence of serotonin, increases approximately 8-fold over promoterless construct or reverse construct controls. Addition of serotonin to the transfected cells results in an additional 2.5–3-fold increase in promoter activity. The selective 5-HT_2 agonist DOI fully replaces native serotonin in inducing transcriptional activation in these assays. Conversely, the selective 5-HT_2 antagonist ketanserin blocks the serotonin-mediated transcriptional activation of the transfected promoter. The extent of the serotonin-mediated induction is consistent with the magnitude of transcriptional activation produced by serotonin, as measured either in Northern analyses (4–5-fold) or in nuclear run-on experiments (approximately 2–3-fold) (6).

The Inr sequence has been shown to play a role in regulating basal promoter activity in numerous promoter regions and to influence the accuracy of transcription initiation (7). In other settings, cooperativity has been observed between the transcription factor SP-1 and Inr-binding protein when the two DNA sequences occur close together in promoters. Inr sequences do not occur near the other putative transcriptional start sites in the 5-HT_2 receptor promoter; this finding is similar to that reported for the human interleukin-1 receptor gene (20). In the 5-HT_2 receptor gene, the distance between the SP-1 site and the Inr is 80 bp; thus, we examined the possibility that such interactions could be important in the 5-HT_2 receptor promoter. To examine the role of the Inr element in the transcriptional activity of the promoter, two short promoter constructs were prepared by PCR. Both constructs contained 75 bp of sequence upstream from the primary transcriptional start site, but one lacked the 8-bp Inr sequence. After ligation into pCAT, transient transfection experiments were performed and transcriptional activity was determined in the presence and absence of DOI. Interestingly the basal, or constitutive, activities of both constructs were the same and similar to that observed with the full length 1.4-kb construct (Fig. 6A). In contrast, however, DOI inducibility was largely lost with either construct (Fig. 6B). Thus, the Inr sequence appears unnecessary either for basal activity or for serotonin-dependent activation by the 5-HT_2 receptor promoter. In addition, the fact that no serotonin-dependent CAT activity was observed with the Inr-containing construct indicates that the sequences responsible for serotonin inducibility lie upstream of the short constructs used in these experiments. A detailed deletional analysis of the 5-HT_2 receptor promoter region is currently in progress, with a view toward identifying such regions. In addition, because the Inr element appears largely unnecessary for the maintenance of basal expression of the promoter, a search for the minimum sequence required for such activity is currently underway. The definition of the *cis* elements necessary for the complex regulation of the expression of the 5-HT_2 receptor gene is an ultimate goal of our studies.

Acknowledgments

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Note Added in Proof

After the review of this manuscript was complete we became aware of the publication of an important paper, relevant to the work presented here (Ding, D., M. Toth, Y. Zhou, C. Parks, B. J. Hoffman, and T. Shenk *Mol. Brain Res.* **20:** 181–191, 1993). This paper reports the cloning and sequence of the 5′-flanking region of the mouse 5-HT_2 receptor promoter region. Many of the response elements are similar or identical to those found by us in the rat. We regret not having been able to discuss these findings in the original text.

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