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Title Page

Characterization of Prejunctional Serotonin Receptors Modulating [^{3}H]-Acetylcholine Release in the Human Detrusor

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Running Title Page

a) **Running title**

Prejunctional 5-HT receptors in the human detrusor

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c)

- Text pages: 9
- Tables: 1
- Figures: 6
- References: 41
- Abstract: 232 words
- Introduction: 394 words
- Discussion: 1308 words

d) **Abbreviations.** OAB, overactive bladder; LUT, lower urinary tract; UI, urinary incontinence; IC, interstitial cystitis; EFS, electrical field stimulation; [^{3}H]-ACh, tritiated acetylcholine; 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine; GR113808A, [1-[2-[(-methylsulphonyl) amino] ethyl]4-piperinidyl]methyl1-methyl-1H-indole-3-carboxylate succinate; SB269970, (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulphonyl)phenol hydrochloride; WAY100635, *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)-cyclohexane-carboxamide trichloride.

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ABSTRACT

Bladder overactivity (OAB) is a chronic and debilitating lower urinary tract (LUT) disorder which affects millions of individuals worldwide. LUT symptoms associated with OAB, such as urgency and urinary incontinence (UI), cause a hygienic and social concern to patients, but their current pharmacological treatment is largely inadequate due to the lack of uroselectivity. Although OAB aetiology remains multifactorial and poorly understood, increasing evidence indicates that serotonin (5-hydroxytryptamine; 5-HT) is an endogenous substance involved in the control of micturition at central and peripheral sites. In this study, we demonstrated the presence of three distinct 5-HT receptors localized at parasympathetic nerve terminals of the human bladder by measuring electrically-evoked tritiated acetylcholine release in isolated detrusor strips. These prejunctional receptors, involved in both positive and negative feed back mechanisms regulating cholinergic transmission, have been characterized by means of 3 highly selective 5-HT antagonists for 5-HT_4, 5-HT_7 and 5-HT_{1A} receptors, namely ([1-[2-[(-methylsulphonyl) amino] ethyl]4-piperinidyl]methyl1-methyl-1H-indole-3-carboxylate) succinate (GR113808A), (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulphonyl)phenol hydrochloride (SB269970) and *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)-cyclohexane-carboxamide trichloride (WAY100635). Under these conditions, we confirmed the facilitatory role of 5-HT_4 heteroreceptors on acetylcholine release and revealed for the first time the occurrence of 5-HT_7 and 5-HT_{1A} heteroreceptors with a facilitatory and an inhibitory action, respectively. Our findings strengthen the novel concept for the use of recently patented selective 5-HT agonists and antagonists for the control of OAB dysfunctions associated with inflammatory conditions, although their therapeutic efficacy needs to be explored in the clinical setting.

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Introduction

Increasing evidence indicates that serotonin (5-hydroxytryptamine; 5-HT) is involved in the control of the micturition process at central and peripheral sites where 5-HT can either reduce or facilitate the lower urinary tract (LUT) functions (Andersson and Pehrson, 2003; Andersson and Wein, 2004; D'Agostino et al., 2004). 5-HT interacts with multiple types of 5-HT receptors which have been divided into subfamilies by convention (Zifa and Fillion 1992; Hoyer et al., 1994). Seven subfamilies have been characterized and some have been further subdivided by overlapping pharmacological properties and second messenger coupling pathway (Raymond et al., 2001). The 5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptors couple to G-proteins, whereas the 5-HT_3 receptors are 5-HT-gated ion channels (Barnes and Sharp, 1999; Hoyer et al., 2002).

Centrally, serotonergic cell bodies in the raphe project to the dorsal horn as well as to autonomic and somatic nuclei of the ventral horn in the lumbosacral spinal cord (de Groat, 2002). These neurones contain 5-HT_2 and 5-HT_{1A} receptors located on different loci at postjunctional or prejunctional sites (Burgard et al., 2003). Collectively, the serotonergic descending pathway is essentially inhibitory.

At variance, peripherally 5-HT induces neuromuscular excitatory effects that have been described in detrusor muscle strips of mouse (Clean et al., 1989), guinea-pig (Messori et al., 1995), rabbit (Barras et al., 1996), pig (Sellers et al., 2000) and man (Tonini et al., 1994). Several *in vitro* studies have shown that the contractile effect of mammalian detrusor in response to 5-HT exposure is mediated by activation of 5-HT_2, 5-HT_3 and 5-HT_4 receptors (Yoshida et al., 2002). In the human detrusor, 5-HT potentiates the neurogenic contractions by activating the 5-HT_4 receptor type (Candura et al., 1996). Since this enhancing effect of 5-HT is antagonized by the muscarinic antagonist atropine and the nerve conduction blocker tetrodotoxin, it was suggested that prejunctional 5-HT receptors located at parasympathetic postganglionic neurons are implicated into the control of acetylcholine (ACh) release (Corsi et al., 1991). Such an assumption, however, was based on postjunctional effects (contractions) only and not verified by assessment of changed of neurotransmitter release. The measurement of the electrically-evoked tritiated ACh (^{3}H-ACh) release in human detrusor strips allowed us: a) to provide direct evidence that 5-HT_4 heteroreceptors are implicated in this stimulation of ACh release; b) to ascertain the involvement of other subtypes of 5-HT receptors in the control of the cholinergic neurotransmission in the human bladder.

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Materials and Methods

Drugs and chemicals. GR113808A, ([1-[2-[(-methylsulphonyl)amino]ethyl]4-piperinidyl]methyl1-methyl-1H-indole-3-carboxylate) succinate and ondansetron were kindly supplied by GSK (Verona, Italy). Other drugs used were 5-carboxamidotryptamine maleate (5-CT) (RBI, Natick, MA, USA), methiothepin maleate (Tocris Cookson Ltd, Bristol, UK). Hemicholinium-3, 5-hydroxytryptamine creatinine sulphate, ketanserin tartrate salt, SB269970 ((*R*)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulphonyl)phenol hydrochloride), WAY100635 (*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)-cyclohexane-carboxamide trichloride) (all from Sigma-RBI, St. Louis, MO, USA) and [methyl-^{3}H] choline (Amersham, Arlington Heights, IL, USA).

Tissue Preparation. Specimens from the anterior part of the urinary bladder dome were obtained from 62 male patients (69 average age) undergoing total cystectomy due to bladder base malignancy. The study was approved by the Ethics Committee of General Hospital of Voghera (Pavia, Italy). Specimens were transported to the laboratory in oxygenated Krebs solution (composition in mM : NaCl 120, KCl 4.7, $MgSO_4$ 0.6, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.0, glucose 10) maintained at 5-6 °C. Muscular strips (20 mm long, 4 mm wide), free of serosal and mucosal layers and with a good alignment of the muscle bundles, were prepared and stored overnight at 4 °C. Strips were then mounted isometrically in 2 ml organ baths superfused with Krebs solution at 37 °C bubbled with 95% O_2 and 5% CO_2. The tissues were allowed to equilibrate for 45 min under a tension of 2 g. Electrical field stimulation (EFS) was applied by means of two platinum electrodes placed parallel to the preparation. From a single surgical specimen various preparations were obtained. Each preparation was used for a single protocol.

Release Experiments. Neuronal release of ^{3}H-ACh was assessed according to the procedure previously described for the human bladder (D'Agostino et al., 2000). Briefly, the preparation was incubated for 45 min with [methyl-^{3}H] choline (74 KBequerel/ml) and stimulated by 10 s pulse trains at 10 Hz (0.2 ms duration, 60 V/cm, 60 s apart) to label neuronal ACh stores. At the end of the labelling period, the preparation was washed out for 120 min by superfusion at a constant rate of 2 ml/min (Minipulse 2HP8 flow inducer, Gilson Medical Electronics, Middleton, WI, USA). Hemicholinium-3 (10 μM) was present in the washout solution throughout the experiment to prevent choline uptake.

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Starting at the 121st min (zero time), superfusion fluid was collected continuously in 3 min periods (6 ml samples). The strip was stimulated 2 times (S_1 and S_2) beginning 9 (S_1) and 54 (S_2) min after zero time. The ^{3}H-outflow was evoked by 150 square wave pulses delivered in 6 trains at 5 Hz (0.5 ms duration, 60 V/cm, 35 s apart). Aliquots (1 ml) of the superfusate were added to 3 ml of Ultima Gold (Packard BioScience, Groningen, The Netherlands) and the tritium content was measured by liquid scintillation spectrometry (Tri-Carb 2700TR, PerkinElmer, Shelton, CT, USA). Quench correction curves were established and external standardization was used for counting efficiency. Both resting and stimulation-evoked outflow of radioactivity was expressed in disintegrations per s (Bequerels) per gram of tissue (Bq/g). The increase of the release caused by stimulation was obtained from the difference between the total tritium outflow during 3 min stimulation plus the following 12 min (stimulation outflow period) and the calculated spontaneous outflow. The decline for the spontaneous outflow was calculated by fitting a linear regression line to the values (expressed in Bq/g) of 3 min-samples before and after the stimulation outflow period. The drugs were added 10 min (5-HT agonists) or 30 min (5-HT antagonists) before the onset of S_2. The prejunctional effect of 5-HT was expressed as S_2/S_1 ratio in comparison to the equivalent ratio obtained in the absence of the drug (control experiments).

The EFS effectiveness in producing neuronal cholinergic smooth muscle contractions (Tonini et al., 1994) were recorded with a force displacement transducer and displayed on a polygraph (Battaglia Rangoni, Bologna, Italy). This allowed us to record simultaneously in each experiment both pre- and postjunctional effects. Postjunctional effects evoked by EFS were assessed as the mean of 6 peak contractions evoked by S_1 and S_2.

Data analysis. Values from individual experiments were averaged, and the S.E.M. values were calculated. Concentration-response curves for prejunctional effects of 5-HT and 5-CT were constructed in the absence and in the presence of 5-HT antagonists. Drug potency estimates were evaluated as -log EC_{50} (negative log of the molar concentration producing half-maximal effect) by nonlinear curve fitting (GraphPad Prism, Version 3.02, GraphPad Software, San Diego, CA, USA). Affinity (pA_2) and apparent affinity (pK_b) estimates were calculated according Schild plot analysis and the method of Furchgott (1972), respectively. The difference between groups of data was evaluated by Student's unpaired t-test. A *p* value less than 0.05 was considered statistically significant.

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Results

In human isolated detrusor strips, EFS produced a contractile response (7.65 ± 1.57 g, n = 27) and a marked ^{3}H-outflow (S_1: 8,354 ± 1,243 Bq/g of dry tissue) (Fig. 1A,C). The contractile effect was cholinergic and neuronal in origin (Tonini et al., 1994) and the ^{3}H-outflow reflected the release of ^{3}H-ACh from neural stores as previously demonstrated (D'Agostino et al., 2000). The S_2/S_1 ratio, 0.805 ± 0.04 in control experiments, was not affected significantly following the exposure to any of the 5-HT antagonists. Conversely, 5-HT (1 nM - 10 μM) affected S_2/S_1 ratio in comparison to control experiments producing a dual concentration-related effect on transmitter release. At concentrations lower than 3 nM, 5-HT slightly but significantly reduced the electrically-evoked ^{3}H-ACh release (-7.9% ± 4.7 at 1 nM; n = 4) (Fig. 2). On the other hand, within 3 nM - 10 μM concentrations range, 5-HT potentiated both contractraction and ^{3}H-ACh release in a concentration-dependent manner with a maximal effect at 10 μM (106 % ± 7.4 compared to control; n=5) (Fig. 1B,D; Fig. 2). A potency (prejunctional pEC_{50} value) of 7.92 ± 0.09 (n = 5; Fig. 2) was calculated. The facilitation of ^{3}H-ACh release paralleled the enhancement of neurogenic contractions (max effect = 96 ± 8.2%; pEC_{50} value of 8.15 ± 0.07).

The facilitatory ^{3}H-ACh release curve of 5-HT was shifted to the right in the presence of 10 - 60 nM GR 113808A, a potent and selective 5-HT_4 antagonist, with a progressive depression of the maximal response (approximately 50% at 60 nM; n = 4, Fig. 2), indicating a non-competitive (functional) antagonism. An apparent affinity value (pK_b) of 9.50 ± 0.05 was estimated.

In the presence of 1 μM methiothepin, a putative 5-$HT_{1/2/3/7}$ receptor antagonist, the prejunctional inhibitory effect observed during the exposure of 1 nM 5-HT was abolished whereas the facilitatory component of the 5-HT curve was not affected (pEC_{50} value of 8.06 ± 0.04, n=5). In such a condition of 5-$HT_{1/2/3/7}$ receptors blockade, GR113808A (3 - 30 nM) was able to antagonize in a competitive manner the facilitatory curve induced by 5-HT (Fig. 3). According to Schild analysis, a pA_2 value of 9.43 ± 0.27 with a slope of 1.06 ± 0.19 (n = 20) was calculated.

In the presence of 3 μM GR113808A, a concentration producing a nearly complete blockade of 5-HT_4 receptors, 5-HT (1 nM - 1 μM) caused a concentration-dependent inhibitory effect on ^{3}H-ACh release with a maximal decrease of 22.58% ± 6.37 (n = 5), and a calculated pEC_{50} of 7.99 ± 0.69. In this condition, the inhibition of the ^{3}H-ACh release was reversed yielding an enhancement (by about 20% at 10 μM concentration of 5-HT) (Fig. 4).

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The putative 5-$HT_{1/7}$ agonist 5-CT, in a 0.1 nM - 1 μM concentration range, caused dual effects on electrically-evoked ^{3}H-ACh release (Fig. 4), similarly to 5-HT (maximal decrease of 23.05% ± 0.45, n = 5) but with an inhibitory potency (pEC_{50} of 9.03 ± 0.05) 10 fold higher than that showed by 5-HT.

Both the inhibitory and facilitatory component of the curve was not affected either in the presence of 100 nM ketanserin, a putative 5-HT_2 antagonist or 10 nM ondansetron, a selective 5-HT_3 antagonist, or 3 μM GR113808A (not shown). SB269970 (0.3 - 30 nM), a new potent and selective 5-HT_7 antagonist, counteracted in a concentration-dependent fashion only the facilitatory component produced by 5-CT (100 nM - 1 μM) (Fig. 5) with a pK_b value of 9.07 ± 0.05 (n = 4).

In condition of 5-HT_7 blockade by 30 nM SB269970, the inhibitory component of the curve produced by 0.1 - 10 nM 5-CT was antagonized in a competitive manner by the selective 5-HT_{1A} antagonist WAY100635 (0.3 - 3 nM) (Fig. 6). The WAY100635 affinity was calculated by Schild plot analysis (pA_2 value 9.81 ± 0.10, slope 0.98 ± 0.01, n = 4).

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Discussion

The modulatory role of 5-HT on parasympathetic function in the human detrusor has been previously investigated in functional experiments dealing with nerve-mediated contractile response to EFS. Under these conditions, a facilitatory role of 5-HT in enhancing cholinergic twitch response through 5-HT_4 receptor activation has been demonstrated (Tonini et al., 1994). This study allowed to demonstrate by means of a direct ^{3}H-ACh release measurement that the modulatory role of 5-HT on parasympathetic nerve terminals innervating the human detrusor is more complex than that previously thought. Indeed, we found that three different 5-HT receptor sites are operative prejunctionally; namely the 5-HT_4, 5-HT_7 and 5-HT_{1A} receptors. The first two receptor types, which are positively coupled to adenylyl cyclase, enhance ^{3}H-ACh release whereas the 5-HT_{1A} subtype, which is coupled to $G_{i/o}$ effector proteins, negatively controls transmitter release. The inhibitory control of ^{3}H-ACh release prevails at subnanomolar concentrations of 5-HT, whereas the facilitatory effect is prominent at higher 5-HT concentrations.

Based on the evidence that the two opposite mechanisms are working simultaneously, the excitatory component was studied after blockade of the inhibitory component, and *vice versa*. In the presence of methiothepin, a 5-HT receptor antagonist for all but the 5-HT_4 type (see Table 1), the facilitatory curve to 5-HT obtained at nanomolar concentrations was shifted to the right in a competitive fashion by the selective 5-HT_4 antagonist GR113808A. The pA_2 affinity value for GR113808A of 9.43 is consistent with its affinity at the 5-HT_4 receptor type reported in the literature (range: 9.0 - 9.7; Gale et al., 1994), thus indicating the involvement of this receptor in the facilitatory mechanism. In the presence of a full blockade of 5-HT_4 receptors (i.e. GR113808A, at 3 μM), but in the absence of methiothepin, 5-HT was able to reveal a concentration-dependent inhibitory effect (max effect: 23% vs. control) which was followed by an excitatory effect at micromolar concentrations unrelated to 5-HT_4 receptors activation.

A dependable receptor analysis of both components of the curve was carried out by means of 5-CT, a 5-$HT_{1/7}$ receptor-preferring agonist, with an affinity/potency higher than 5-HT (see Table 1). 5-CT caused a biphasic curve characterized by an inhibitory component followed by an excitatory component. The biphasic response caused by 5-CT was unaffected by ketanserin, ondansetron and GR113808A, thus excluding the involvement of 5-HT_2, 5-HT_3 and 5-HT_4 receptors (see Table 1 for antagonist receptor selectivity). Conversely, in the presence of SB269970, a potent and selective 5-HT_7 antagonist (Lovell et al., 2000; Thomas et al., 2000), the excitatory component of the curve was

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concentration-dependently inhibited up to the suppression at 30 nM. The calculated apparent affinity value (pK_b) of 9.07 indicates that the second excitatory component occurring at high 5-HT/5-CT concentrations is mediated by the 5-HT_7 receptor type. In the presence of 5-HT_7 receptor blockade, a pure 5-CT-mediated inhibitory curve was observed. This curve was concentration-dependently antagonized in a competitive manner by WAY100635, a selective 5-HT_{1A} receptor antagonist (Forster et al., 1995). The calculated affinity value (pA_2 of 9.81) characterized unambiguously the inhibitory 5-HT receptor as the 5-HT_{1A} subtype.

Our findings raise the intriguing possibility that 5-HT differentially regulates, *via* distinct 5-HT heteroreceptors, ACh release from parasympathetic nerve terminals which, in turn, affects detrusor smooth muscle contraction. However, the importance of these 5-HT receptors in regulating cholinergic nerve activity and bladder tone in physiological conditions is presently unknown. In the bladder, the serotonergic innervation is scanty (de Groat and Booth, 1993; Hoyle and Burnstock, 1993) and therefore is unlikely that neuronal 5-HT may substantially contribute to the regulation of parasympathetic function physiologically. This is in agreement with our results regarding the inefficacy of various 5-HT antagonists on ACh release. Conversely, the discovery of a double positive serotonergic feed-back mechanism together with a negative one, regulating the release of ACh, may have profound implications in the pathophysiological mechanisms underlying bladder disorders and therapy. In fact, since the 5-HT_4/5-HT_7 -mediated potentiation of cholinergic transmission is experimentally prevalent when the levels of the agonist in the neural cleft are elevated, one can speculate that 5-HT might play a role in pathological conditions associated with enhanced turnover rates or increased levels from tissue sources. Although endogenous 5-HT is synthesized mostly by enterochromaffin cells in the gastrointestinal tract and, to a lesser extent, in the central and peripheral nervous system, inflammatory cells including mast cells are possible sources of 5-HT (Theoharides et al., 1982; Ford and Kava, 1997). It is noteworthy that mast cells are universally found in close proximity to nerves, where factors released from mast cells and neurotransmitters from nerves (i.e. ACh) (Spanos et al., 1996) are involved in a bidirectional communication, promoting also axon reflex via local ganglia to the spinal cord and then to the brain (Maurer et al., 2003). In this respect, a well documented mastocytosis within the muscle bundles and in close proximity to bladder nerve terminals has been detected under pathological conditions associated with interstitial cystitis (IC), a sterile bladder inflammation of unknown aetiology (Theoharides et al., 2001). IC is characterized by a variety of OAB symptoms which include urinary frequency and urgency (Sant and Theoharides, 1999;

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Chancellor and Yoshimura, 2004). It is conceivable to assume that these symptoms, partly related to parasympathetic activation, are due to 5-HT released by mast cell degranulation, since in the light of our results elevated levels of 5-HT cause a marked rise in the release of ACh, the most important excitatory transmitter in the human detrusor. Indeed, an altered control of peripheral cholinergic pathway is currently regarded as a key factor in the pathophysiology of OAB and urinary incontinence (UI) (Chess-Williams, 2002; Andersson and Wein, 2004; Fry et al., 2004).

With respect to the implication of 5-HT receptors in OAB and UI pathophysiology, the 5-HT_4 agonist cisapride can cause UI (Boyd and Rohan, 1994) as well as an improvement of bladder function in patients with bladder voiding defects associated with detrusor hypocontractility (Franceschetti et al., 1996). A modified 5-HT_4 receptor-mediated response was observed in detrusor muscle strips of patients with different types of OAB (Mundy et al., 1994; Chapple et al., 2004), but no clinical data regarding the efficacy of 5-HT_4 selective antagonists on neurogenic and non-neurogenic bladder dysfunctions are available, as well as information regarding the involvement or alteration of 5-HT_7 receptors. Based on our results, however, selective antagonists at 5-HT receptors can be proposed for the treatment of micturition disturbances associated with detrusor hyperactivity, since 5-HT_4 and 5-HT_7 receptors have been shown to potentiate cholinergic activity at both peripheral (this study) and supraspinal sites (D'Agostino et al., 2004).

In addition, our study provides new insights into the use of selective 5-HT_{1A} agonists in LUT disorders. Currently, these agents are considered potentially useful in the treatment of stress UI, a condition in which incontinence episodes are associated with a reduced outlet control. Although these compounds seem to exert their prevalent action on outlet resistance at spinal cord sites in the Onuf's nucleus (Thor, 2003; Thor and Donatucci, 2004), our results suggest an additional site of action. In fact, the activation of 5-HT_{1A} inhibitory heteroreceptors on cholinergic terminals might contribute to increase bladder capacity by reducing the cholinergic drive to the detrusor. This may represent an additional mechanism to prevent involuntary leakages due to sphincter incompetence and, in turn, stress UI. The same peripheral mechanism might be partly involved in the increased bladder capacity caused by 8-OH-DPAT, a 5-HT_{1A} agonist, in cats with spinal cord injury (Gu et al., 2004).

In conclusion, our findings provide the first evidence for the presence of peripheral excitatory 5-HT_7 heteroreceptors other than 5-HT_4 receptors and of the inhibitory 5-HT_{1A} subtype that control parasympathetic drive in the human detrusor. The present study strengthens the novel concept for the use of recently patented selective 5-HT agonists and antagonists for the control of OAB dysfunctions

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(D'Agostino et al., 2004), although their therapeutic efficacy needs to be explored in the clinical setting.

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Footnotes

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b) Reprint request to:

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Legends for figures

Fig. 1. Time course of electrically-evoked contractile responses (upper panels) and ^{3}H-ACh outflows (lower panels) in human detrusor strips in control conditions (panel A and C) and in the presence of 1 μM 5-HT (panel B and D). In panel C and D, each point represents the radioactivity per gram of tissue in the superfusate collected in 3-min samples.

Fig. 2. Effects of 5-HT on electrically-evoked ^{3}H-ACh outflow in human detrusor strips in the absence (○) and in presence of GR113808A, a selective 5-HT$_4$ receptor antagonist, at 1 nM (♦), 10 nM (◊) and 60 nM (▲). Given are the means ± S.E.M. of 3-7 experiments.

Fig. 3. ACh release experiments in human detrusor strips in the presence of 1μM methiothepin, a putative 5-HT$_{1/2/3/7}$ receptor antagonist. Effect of 5-HT on electrically-evoked ^{3}H-ACh outflow in the absence (○) and in presence of GR113808A, a selective 5-HT$_4$ receptor antagonist, at 3 nM (■), 10 nM (◊) and 30 nM (●). Given are the means ± S.E.M. of 5 experiments.

Fig. 4. Effect of 5-CT (○) and of 5-HT (■) on electrically-evoked ^{3}H-ACh outflow in human detrusor strips. Trials for 5-HT were carried out in the presence of a complete 5-HT$_4$ receptor blockade by 3 μM GR113808A. Given are the means ± S.E.M of 5 experiments.

Fig. 5. ACh release experiments in human detrusor strips in the presence of a complete 5-HT$_4$ receptor blockade by 3 μM GR113808A. Effect of 5-CT on electrically-evoked ^{3}H-ACh outflow in the absence (○) and in presence of SB269970, a selective 5-HT$_7$ receptor antagonist, at 0.3 nM (♦), 1 nM (◊), 10 nM (▲) and 30 nM (□). Given are the means ±S.E.M. of 4 experiments.

Fig. 6. ACh release experiments in human detrusor strips in the presence of a complete 5-HT$_4$ and 5-HT$_7$ receptor blockade by 3 μM GR113808A and 30 nM SB269970, respectively. Effect of 5-CT on electrically-evoked ^{3}H-ACh outflow in the absence (○) and in presence of WAY100635, a selective 5-HT$_{1A}$ receptor antagonist, at 0.3 nM (▲), 1nM (□) and 3nM (♦). Given are the means ± S.E.M. of 4 experiments.

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Tables

Table 1. Comparison of affinity (pA_2 - pK_i) estimates of 5-HT ligands at native 5-HT receptor subtypes

5-HT ligands	5-HT_{1a}	5-HT_{1b}	5-HT_{1d}	5-HT_{2a}	5-HT_{2c}	5-HT_3	5-HT_4	5-HT_7
5-HT	**8.1 - 8.7**	**-**	**-**	**5.5 - 6**	**-**	**6 - 6.9**	**7**	**8.2 - 8.5**
5-CT	**8.6 - 9.5**	**7.9**	**8.1**	**3.5 - 4.7**	**5.7**	**inactive**	**5.5**	**9.1 - 9.5**
Methiothepin	**7.1 - 7.7**	**8.1**	**7.44 - 7.7**	**8.7 - 9.40**	**8.2**	**7.5 - 8.5**	**inactive**	**8.5 - 9**
Ketanserin	**5.7 - 5.9**	**5.7**	**-**	**9.4 - 9.3**	**6.5**	**7 - 8.5**	**inactive**	**6.1 - 6.7**
GR113808	**<5**	**<5**	**<5**	**-**	**-**	**6**	**9.0 - 9.7**	**-**
SB269970	**< 5**	**6**	**< 6**	**< 5**	**<5**		**5.9**	**9**
Ondansetron	**-**	**-**	**-**	**-**	**-**	**8.0 - 8.6**	**<<5**	**-**
WAY100635	**9 - 9.7**	**-**	**-**	**-**	**-**	**7.9 - 9.0**	**-**	**5.5**

Affinity (pA_2 - pK_i) estimates from literature: Zifa and Fillon, 1992; Hoyer et al., 1994; Lovell et al., 2000; Thomas et al., 2000; Forster et al., 1995.

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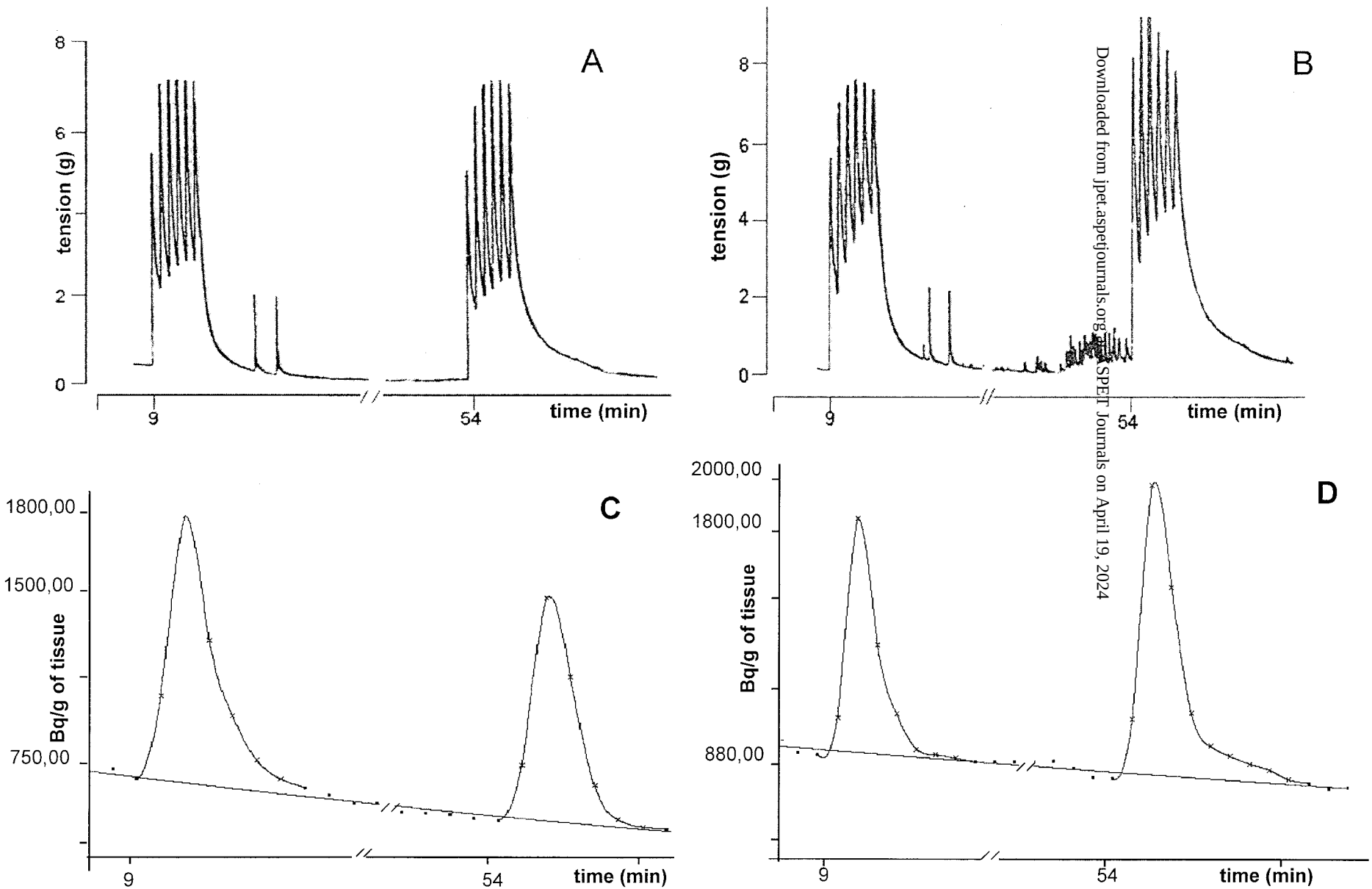

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FIGURE 1

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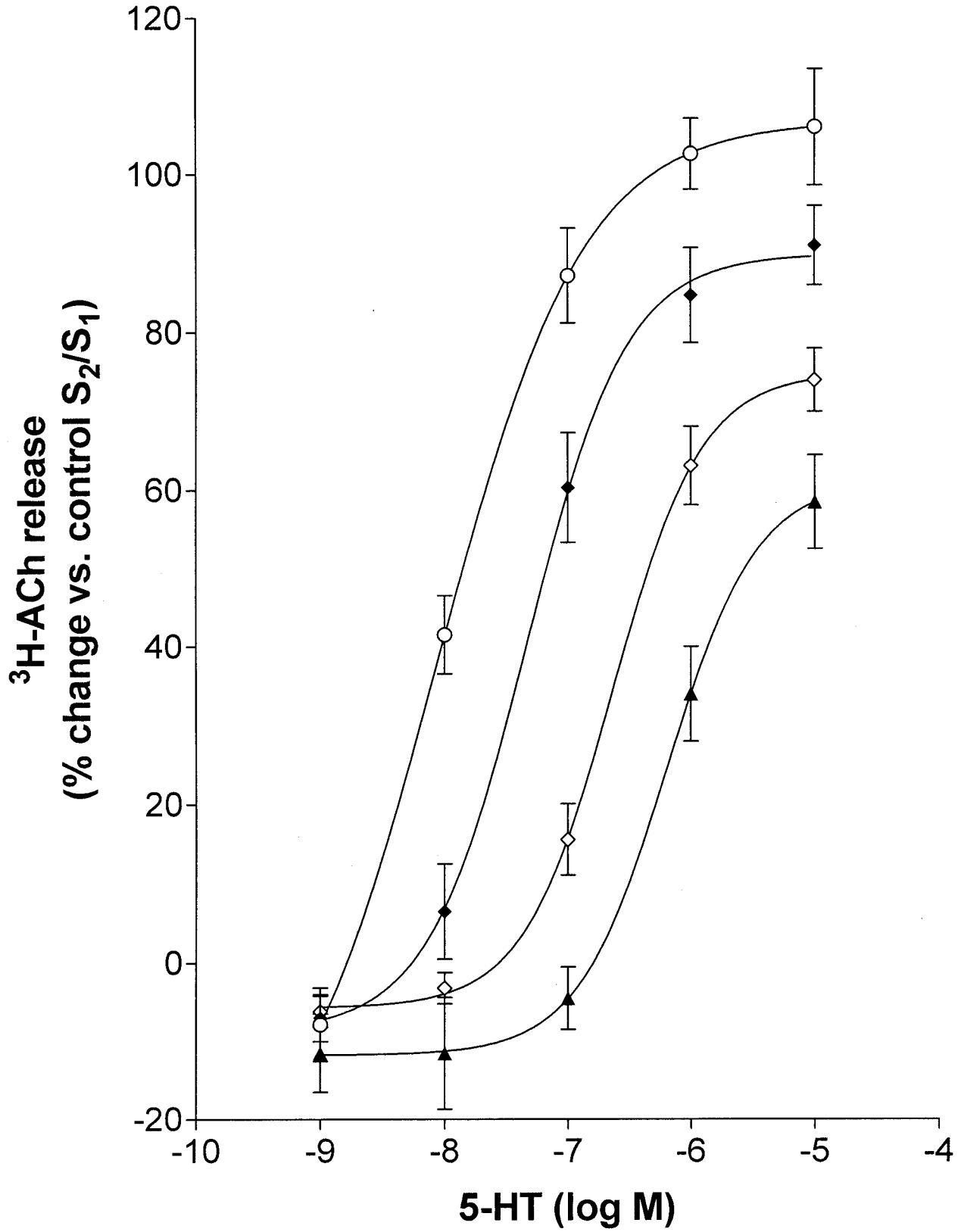

FIGURE 2

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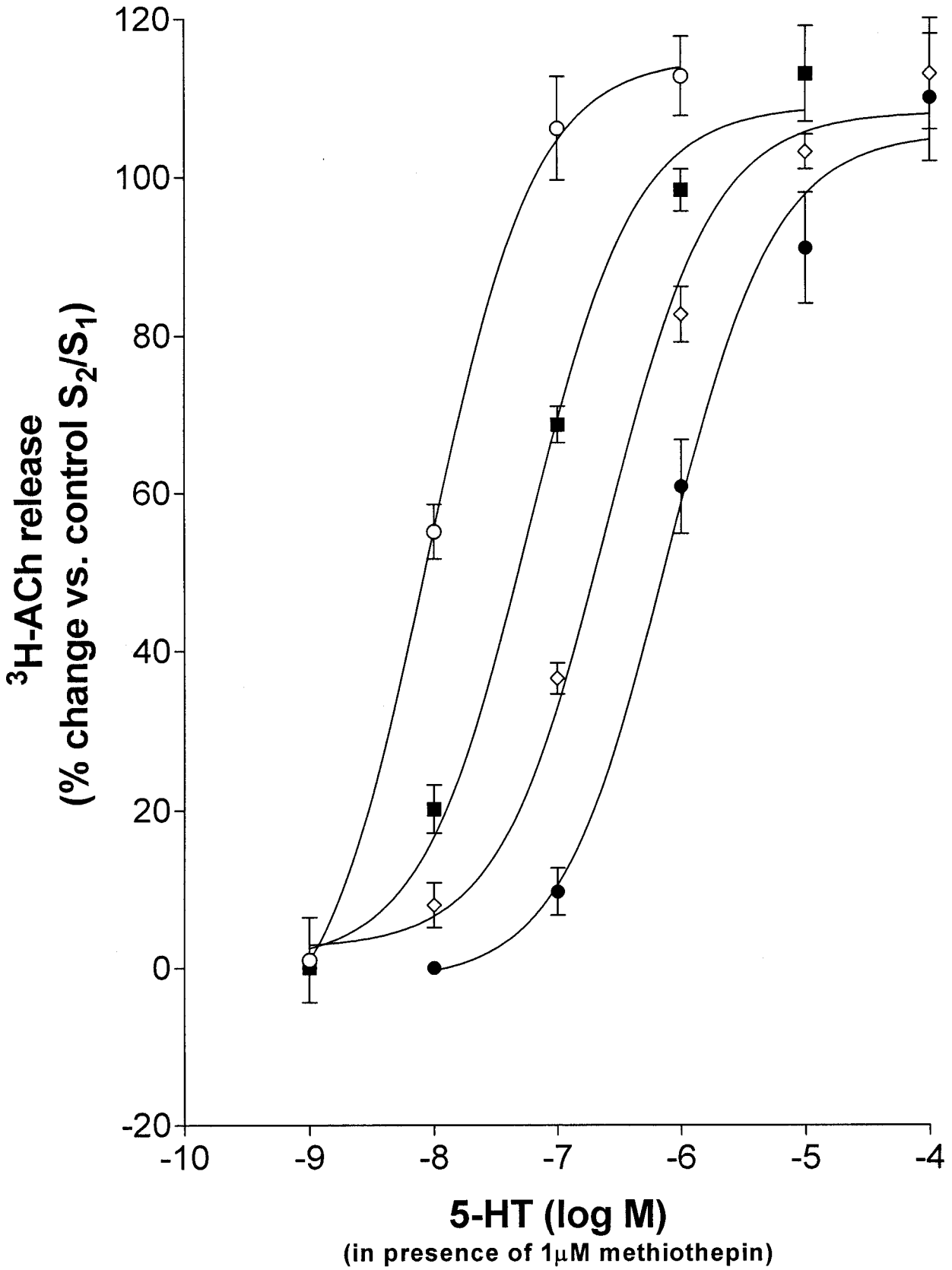

FIGURE 3

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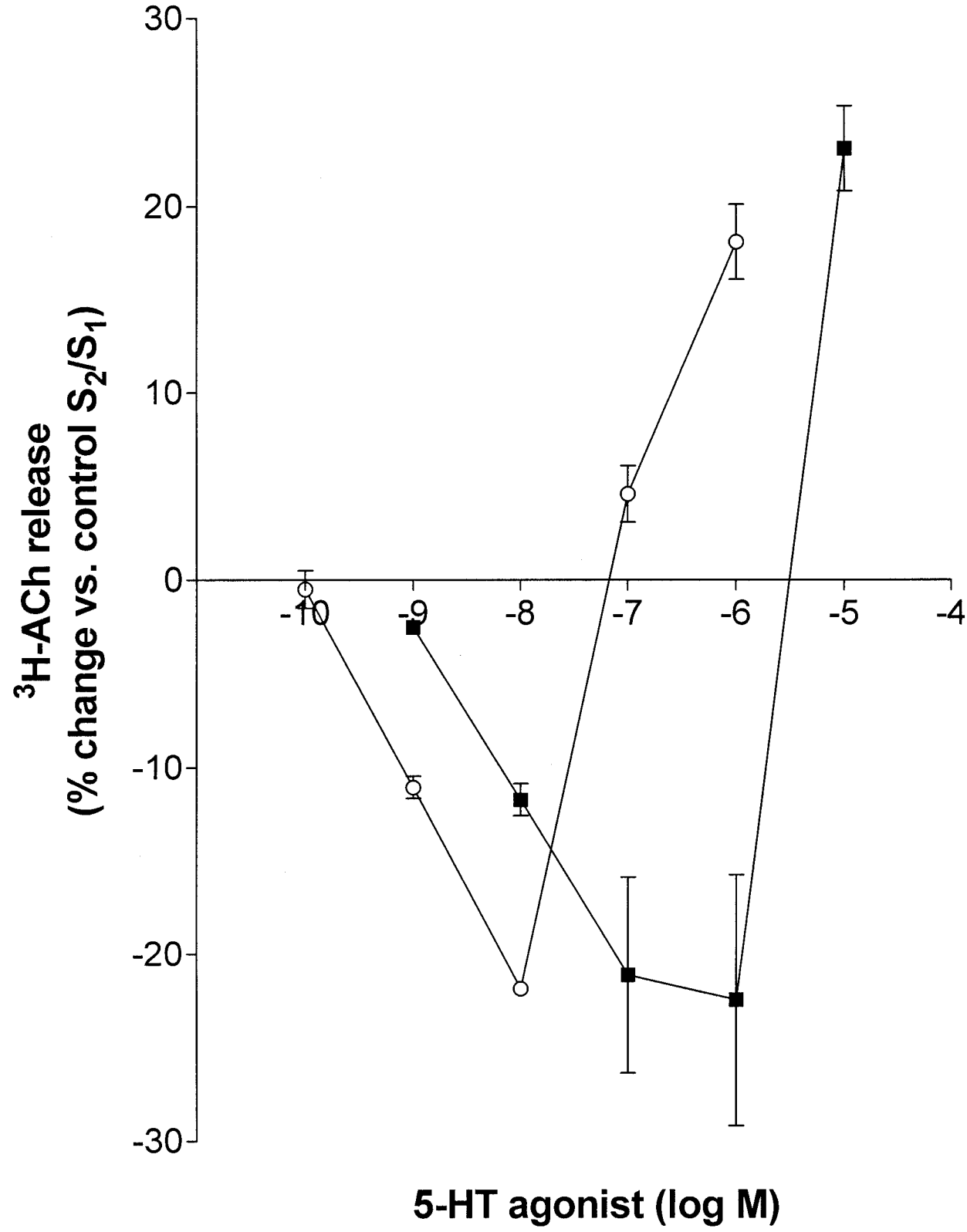

FIGURE 4

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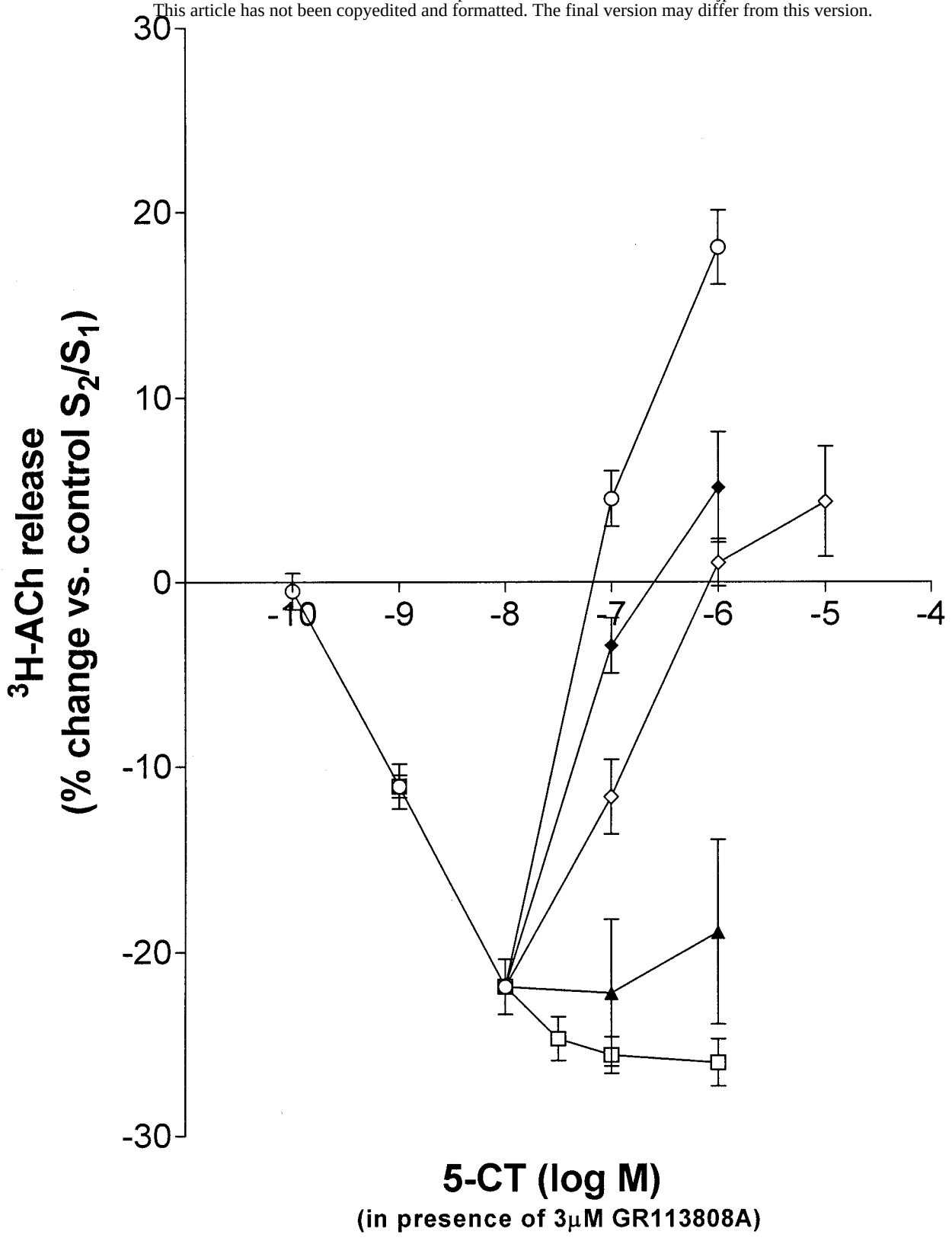

FIGURE 5

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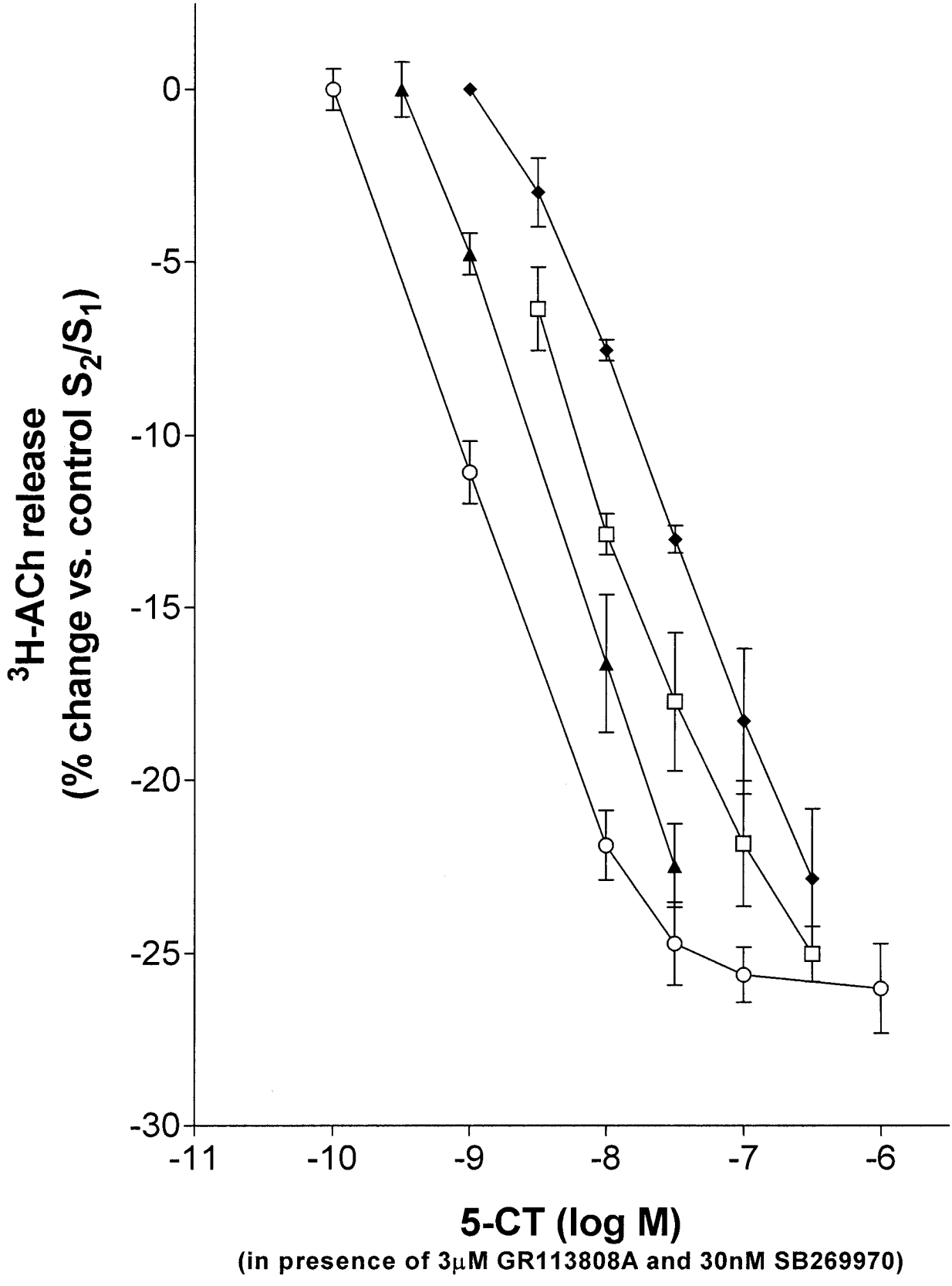

FIGURE 6

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