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Regulation of [^{3}H]norepinephrine release from guinea pig hippocampus by σ_2 receptors

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Abstract

The binding profile of the σ_2 receptor ligand endo-*N*-(8-methyl-8-azabicyclo[3.2.1.]oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1*H*-benzimidazole-1-carboxamidehydrochloride (BIMU-8) had previously been determined, but its agonist/antagonist status at σ_2 receptors had not been identified. We therefore investigated the effects of BIMU-8 for its ability to regulate the stimulated release of [^{3}H]norepinephrine from slices of guinea pig hippocampus. BIMU-8 alone, at a concentration chosen to occupy 50% of σ_2 receptors, had no significant effect on *N*-methyl-D-aspartate (NMDA)-stimulated release of [^{3}H]norepinephrine. We have shown previously that the σ receptor agonist (+)-pentazocine inhibits NMDA-stimulated release in a concentration-dependent manner, producing a biphasic inhibition curve. Similarly, the σ receptor agonist 1*S*,2*R*-(−)-*cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine (BD737) produced a broad inhibition curve. The inhibition by low concentrations of (+)-pentazocine or BD737 that selectively activated σ_1 receptors was reversed by the σ_1-selective receptor antagonist (1-(cyclopropylmethyl)-4-2′-oxoethyl)piperidine HBr (DuP 734). In the current study, when the σ_1 component of inhibition by (+)-pentazocine was blocked by DuP 734, the remaining component of inhibition mediated by σ_2 receptors was reversed by BIMU-8. Our results suggest that (1) BIMU-8 is an antagonist at σ_2 receptors and that (2) σ_2 receptors contribute to regulation of norepinephrine release in guinea pig hippocampus.

Keywords: σ_2 Receptor; Norepinephrine; Hippocampus; BIMU-8; (+)-Pentazocine

1. Introduction

In a study by Martin et al. (1976), σ receptors were named as the receptor through which the benzomorphan *N*-allylnormetazocine (SKF10,047) produced its psychotomimetic effects in the chronic spinal dog. Later, the demonstration that the effects of SKF10,047 were not reversible by naltrexone (Vaupel, 1983) indicated that the σ receptor did not belong to the opioid receptor family. Although much has been learned about the pharmacology and localization of σ receptors in the central nervous system since their original identification (for review, see Walker et al., 1990), their physiological roles remain elusive.

The function(s) of σ receptors in different brain regions has often been inferred from their localization (Gundlach et al., 1986; McLean and Weber, 1988). For example, σ receptors may be important in motor function because they are localized in motor regions, and may be involved in maintenance of emotional well-being, because they are localized in limbic regions (Gundlach et al., 1986). One limbic brain area with an appreciable number of σ receptors is the hippocampus (Mash and Zabetian, 1992). Recent studies suggest that σ receptors in the hippocampus modify *N*-methyl-D-aspartate (NMDA)-induced currents (Monnet et al., 1992; Bergeron et al., 1995) and modify the release of acetylcholine (Junien et al., 1991) and norepinephrine (Gonzalez-Alvear and Werling, 1995) from hippocampal slices. A recent study also provides evidence that σ receptor ligands may enhance NMDA-stimulated norepinephrine release from hippocampal slices under certain conditions (Monnet et al., 1996). The enhancement reported by these investigators, however, appears not to be mediated by σ_1, σ_2 or σ_3 receptors.

Recent evidence for more than one type of σ receptor has led to further characterization of the function of these subtypes (Quirion et al., 1992). There are at least two σ receptor subtypes, recently designated as σ_1 and σ_2 recep-

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tors. The development of compounds selective for σ_1 receptors has enabled further identification of σ receptor properties in functional assays (Bowen et al., 1988). (+)-Pentazocine is a relatively selective σ_1 receptor agonist with an affinity of 2–6 nM for the σ_1 receptor and about 450 nM for the σ_2 receptor in radioligand binding studies (Rothman et al., 1990; Connor and Chavkin, 1992). Therefore, at concentrations below 100 nM, one can assume most physiological effects of (+)-pentazocine are via the σ_1 receptor. This has been confirmed in recent studies using the σ_1-selective receptor antagonist (1-(cyclopropylmethyl)-4-2′-oxoethyl)piperidine HBr (DuP 734) (Culp et al., 1992; Tam et al., 1992). This compound reversed completely the inhibition of [^{3}H]dopamine release from rat striatum (Gudelsky, 1995) and of [^{3}H]norepinephrine release from rat hippocampus (Gonzalez-Alvear and Werling, 1995) produced by 100 nM (+)-pentazocine. Also, 1*S*,2 *R*-(−)-*cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine (BD737) is a relatively selective σ_1 receptor agonist with K_D values for σ_1 and σ_2 receptors of about 1–2 nM (Bowen et al., 1992) and 500 nM (Rothman et al., 1990), respectively.

Given the evidence which suggests important roles for σ receptors in the hippocampus, and since both σ receptor subtypes have been localized to this region (Connor and Chavkin, 1992), we sought to examine whether both σ receptor subtypes might function to regulate norepinephrine release from hippocampal slices. In our initial study, we demonstrated that both σ_1 and σ_2 receptors contribute to regulation of [^{3}H]norepinephrine release by (+)-pentazocine and BD737 in rat hippocampal slices (Gonzalez-Alvear and Werling, 1995). Unfortunately, at the time of those experiments, we had not been able to obtain selective σ_2 receptor ligands to examine further the role of σ_2 receptors in release. The compound endo-*N*-(8-methyl-8-azabicyclo[3.2.1.]oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1*H*-benzimidazole-1-carboxamidehydrochloride (BIMU-8) was reported to have high selectivity for σ_2 receptors in binding assays (Bonhaus et al., 1993), but had not been tested in a functional assay of σ-receptor activity. When BIMU-8 became available, we therefore tested it for potential effects on σ receptor agonist-mediated inhibition of [^{3}H]norepinephrine release in guinea pig hippocampus.

2. Materials and methods

2.1. Chemicals

The following chemicals and reagents were kindly provided by or obtained from the following sources: 1*S*,2 *R*-(−)-*cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine (BD737) (Drs. Wayne Bowen and Brian de Costa, NIDDK, National Institutes of Health, Bethesda, MD, USA); BIMU-8 (Dr. Doug Bonhaus, Roche Bioscience, Palo Alto, CA, USA); desipramine hydrochloride, NMDA and yohimbine hydrochloride (Research Biochemicals International, Natick, MA, USA); (1-(cyclopropylmethyl)-4-2′-oxoethyl)piperidine HBr (DuP 734) (Dr. Rob Zaczek, Du Pont Merck Pharmaceutical, Wilmington, DE, USA); [^{3}H]norepinephrine (spec. act. 42 Ci/mmol, Amersham, Arlington Heights, IL, USA); and (+)-pentazocine (Research Technology Branch, NIDA, Rockville, MD, USA).

2.2. [^{3}H]Norepinephrine release experiments

Release of [^{3}H]norepinephrine was determined using procedures established in this laboratory (Gonzalez-Alvear and Werling, 1994). All experiments were performed under the guidelines and with the approval of the George Washington University Institutional Animal Care and Use Committee. Adult male Hartley guinea pigs (200–400 g, Hilltop, Scottdale, PA, USA) were housed in the animal facility at the George Washington University and provided with food and water ad libitum. Guinea pigs were killed by decapitation and the brains were removed to ice. Hippocampi were dissected and chopped into 250 × 250 μm strips with a Sorvall T-2 tissue sectioner. Tissue slices were suspended in oxygenated modified Krebs-HEPES buffer (in mM, 127 NaCl, 5 KCl, 1.3 NaH_2PO_4, 2.5 $CaCl_2$, 15 HEPES, 10 glucose; pH adjusted to 7.4 with NaOH) by trituration through a plastic transfer pipette. For NMDA stimulation, Mg^{2+} was always omitted from the buffer since this ion blocks conductance through the NMDA-operated cation channel, which must be operable to produce release using this stimulus. Buffers were oxygenated throughout the experiments. Tissue slices were washed three times in buffer, resuspended in 20 ml buffer and incubated with 50 nM [^{3}H]norepinephrine and 0.1 mM ascorbic acid for 30 min. The tissue was then washed twice in 20 ml buffer and once in buffer containing 1 μM desipramine and 1 μM yohimbine. These two drugs were included in all subsequent steps of the experiment to prevent reuptake of and feedback inhibition by the released [^{3}H]norepinephrine. Tissue was suspended a final time in buffer and distributed in 275 μl aliquots between glass-fiber filter discs into chambers of a Brandel (Gaithersburg, MD, USA) superfusion apparatus. Buffer was superfused over the tissue at a flow rate of 0.6 ml/min. A low, stable baseline release of approximately 0.9%/min was established over a 30 min period. Tissue was then stimulated to release [^{3}H]norepinephrine by a 2 min exposure to 100 μM NMDA (stimulus 1). The inflow was then returned to a non-stimulating buffer (interstimulus interval) for a period of 10 min. If a potential inhibitor of release was being tested, it was introduced during this time. The tissue was then stimulated a second time for 2 min with 100 μM NMDA in the presence or absence of inhibitor as appropriate (stimulus 2). Inflow was again returned to non-stimulating buffer to allow re-establishment of baseline release. Radioactivity remaining in the tissue was then extracted by

a 45 min exposure to 0.2 N HCl. Superfusates were collected at 2-min intervals in scintillation vials, and released radioactivity was determined by liquid scintillation spectroscopy.

In each experiment, each treatment was repeated in triplicate. Since each experiment was performed using a superfusion apparatus that contained 18 chambers, six treatments could be assessed in a single experiment. A control treatment in which tissue was stimulated with NMDA without any potential modulator of stimulated release was included in each experiment. Data were expressed as radioactivity released above baseline during the collection interval (fractional release, %) or as a percentage of total radioactivity released by a 2 min exposure to 100 μM NMDA (% control stimulated release). In experiments on inhibition of release by various drugs, all data were statistically analyzed as ratios (stimulus 2/stimulus 1) before transformation of data into % control stimulated release. Data are presented as % control stimulated release for facility in comparison across experiments. All statistical analyses were performed by two-way factorial analysis of variance and post-hoc, two-tailed Dunnett's test as indicated. Statistical significance was accepted at P values < 0.05.

3. Results

Release stimulated by a 2-min exposure to 100 μM NMDA was $2.9 \pm 0.3\%$ ($n = 10$) of total radiolabelled norepinephrine contained in the tissue at the initiation of the stimulus. The σ receptor agonist BD737 was tested for its ability to affect release of [^{3}H]norepinephrine stimulated by NMDA. BD737 produced a concentration-related inhibition of NMDA-stimulated [^{3}H]norepinephrine release over a concentration range of 0.1–100 nM (Fig. 1).

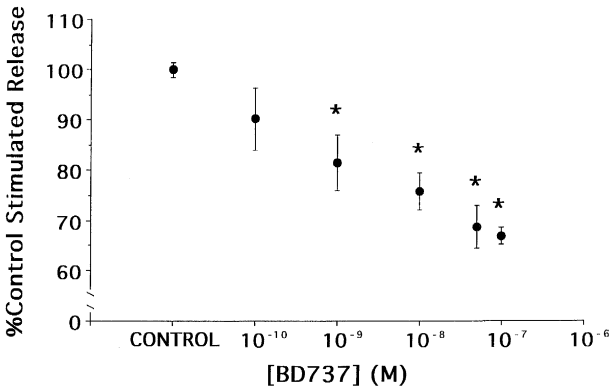

Fig. 1. Effects of BD737 on NMDA-stimulated [^{3}H]norepinephrine release from slices of guinea pig hippocampus. Data are expressed as % release stimulated by 100 μM NMDA (% control release) in the presence of the indicated concentration of BD737. For details of analysis, see Section 2. * Inhibition of stimulated [^{3}H]norepinephrine release significantly different from control by ANOVA and post-hoc Dunnett's (two-tailed; $P < 0.05$) ($n = 3$ independent experiments for each concentration of BD737, and 7 for control).

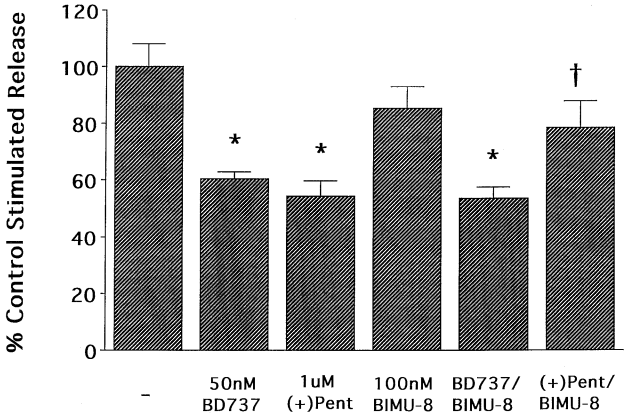

Fig. 2. Effects of BIMU-8 on σ agonist-mediated inhibition of NMDA-stimulated [^{3}H]norepinephrine release from guinea pig hippocampal slices. Release was stimulated by 100 μM NMDA in the presence or absence of a σ agonist (BD737 (50 nM) or (+)-pentazocine (1 μM)) and/or BIMU-8 (100 nM) as indicated. Data are expressed as % of release stimulated by NMDA alone (first column; % control stimulated release). * Significantly different from no inhibitor (NMDA alone) by ANOVA and post-hoc Dunnett's (two-tailed; $P < 0.05$). † Significantly different from inhibition by (+)-pentazocine alone by post-hoc Dunnett's (two-tailed; $P < 0.05$) ($n = 5$ independent experiments for each treatment).

BD737 inhibited approximately 35% of stimulated release, with an apparent IC_{50} of about 1 nM. No clear plateau was visible over this range, but the relative affinities of BD737 for σ_1 and σ_2 receptors of about 1–2 nM (Bowen et al., 1992) and 500 nM (Rothman et al., 1990), respectively, suggested that both receptors might be contributing to the observed response at the higher concentrations.

The σ_2 receptor ligand BIMU-8 (K_i for $\sigma_2 = 20$ nM; Bonhaus et al., 1993) was first tested for effects on NMDA-stimulated and on basal [^{3}H]norepinephrine release in the absence of BD737 or (+)-pentazocine. We chose a concentration of 100 nM BIMU-8, anticipating that this concentration would produce a nearly complete occupation of σ_2 receptors without any significant occupation of σ_1 receptors (K_i at $\sigma_1 = 6.9$ μM; Bonhaus et al., 1993). At a concentration of 100 nM, BIMU-8 neither stimulated nor reduced release with respect to baseline, nor did it have a significant effect on release stimulated by 100 μM NMDA ($85.6 \pm 7.5\%$ S.E.M. of NMDA-stimulated release, $n = 5$) (Fig. 2).

We then tested BD737 at a 50 nM concentration, which should primarily occupy σ_1 receptors, and (+)-pentazocine at a 1 μM concentration which should occupy the majority of both σ_1 and σ_2 receptors. Both BD737 and (+)-pentazocine produced significant inhibition of NMDA-stimulated [^{3}H]norepinephrine release, confirming our earlier studies that identified both subtypes of σ receptors as regulators of NMDA-stimulated release (Gonzalez-Alvear and Werling, 1994, 1995). To investigate whether BIMU-8 might act as an antagonist at σ_2 receptors, we also tested it at a 100 nM concentration against the inhibition of stimulated release produced by 50

nM BD737 and 1 μM (+)-pentazocine. In the presence of BIMU-8, no reversal of BD737-mediated inhibition was observed, but a significant reversal of inhibition mediated by 1 μM (+)-pentazocine was demonstrated (Fig. 2).

We then tested a range of concentrations of BIMU-8 to examine the concentration dependency of reversal of inhibition by BIMU-8 as a σ_2 receptor antagonist. We tested BIMU-8 over a concentration range from 5 nM to 100 nM, based on the reported affinity of BIMU-8 for σ_2 receptors ($K_i = 20$ nM; Bonhaus et al., 1993). In these experiments, we included a 100 nM concentration of the σ_1-selective receptor antagonist DuP 734 to eliminate any contribution of inhibition by (+)-pentazocine acting at σ_1 receptors. We chose a 100 nM concentration of DuP 734 (K_i for $\sigma_1 = 10$ nM; Culp et al., 1992), anticipating that this concentration would produce a greater than 90% occupation of σ_1 receptors. Over this range of concentrations, BIMU-8 displayed a concentration-dependent reversal of (+)-pentazocine-mediated inhibition of NMDA-stimulated [^{3}H]norepinephrine release, with a significant reversal achieved at the 100 nM concentration of BIMU-8. Stimulated release in the presence of 100 nM BIMU-8 alone was not significantly different from control stimulated release (Fig. 3).

We then tested 100 nM BIMU-8 for its ability to antagonize inhibition of NMDA-stimulated [^{3}H]norepinephrine release produced by a range of concentrations of (+)-pentazocine. We tested BIMU-8 against concentrations of (+)-pentazocine (500 nM–5 μM) that should produce a complete occupation of σ_2 receptors. In these experiments, we also included a 100 nM concentration of DuP 734 to eliminate any contribution of inhibition by (+)-pentazocine acting at σ_1 receptors. Under these conditions, 100 nM BIMU-8 was able to significantly reverse inhibition of NMDA-stimulated release mediated by 500 nM–5 μM (+)-pentazocine (Fig. 4). Release in the presence of BIMU-8 and this concentration range of (+)-pentazocine was not significantly different from control NMDA-stimulated release (Fig. 4).

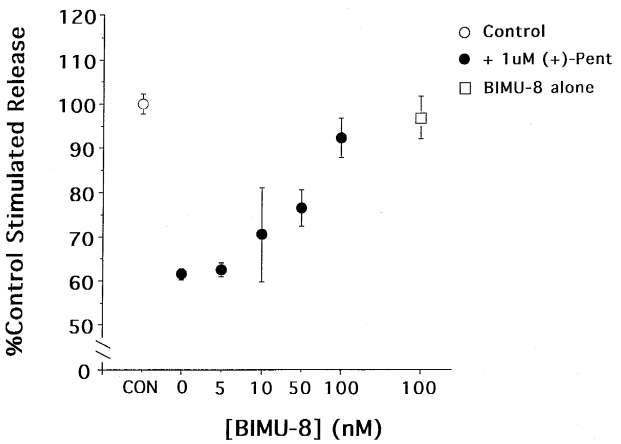

Fig. 3. Effects of increasing concentrations of BIMU-8 on (+)-pentazocine-mediated inhibition of NMDA-stimulated [^{3}H]norepinephrine release from guinea pig hippocampal slices. Release was stimulated by 100 μM NMDA in the presence of DuP 734 (100 nM) (control; open circle), or NMDA and DuP 734 in the presence of BIMU-8 (5–100 nM)+(+)-pentazocine (1 μM) (closed circles) or BIMU-8 (100 nM) alone (open square). Data are expressed as a % of release stimulated by NMDA in the presence of DuP 734 (% control stimulated release). Inhibition of stimulated release by (+)-pentazocine (1 μM) alone was significantly different from control by ANOVA and post-hoc Dunnett's (two-tailed; $P < 0.05$). Inhibition by 1 μM (+)-pentazocine in the presence of 100 nM BIMU-8 was significantly different from inhibition by 1 μM (+)-pentazocine alone by post-hoc Dunnett's (two-tailed; $P < 0.05$) ($n = 3$ independent experiments for each treatment).

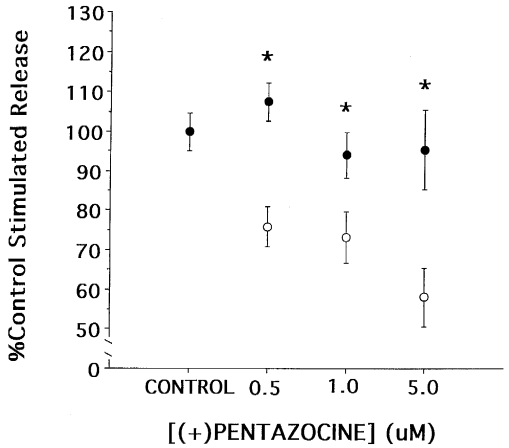

Fig. 4. Effects of BIMU-8 on (+)-pentazocine-mediated inhibition of NMDA-stimulated [^{3}H]norepinephrine release from guinea pig hippocampal slices. Release was stimulated by 100 μM NMDA in the presence of DuP 734 (100 nM) (control), or NMDA and DuP 734 in the presence of the indicated concentration of (+)-pentazocine alone (open circles) or with the addition of BIMU-8 (100 nM) (closed circles). Data are expressed as a % of release stimulated by NMDA in the presence of DuP 734 (% control stimulated release). Inhibition of stimulated release by (+)-pentazocine (0.5–5.0 μM) alone was significantly different from control by ANOVA and post-hoc Dunnett's (two-tailed; $P < 0.05$) ($n = 3$ independent experiments for each treatment). * Inhibition by (+)-pentazocine in the presence of BIMU-8 (closed circles) was significantly different from inhibition by (+)-pentazocine alone (open circles) by post-hoc Dunnett's (two-tailed; $P < 0.05$) ($n = 3$ independent experiments for each concentration of (+)-pentazocine and (+)-pentazocine in the presence of BIMU-8, and $n = 6$ for control).

4. Discussion

The recent characterization of σ receptor subtypes has led to further insight into their possible functions in different brain regions (Quirion et al., 1992; Vilner et al., 1995). In general, σ_1 receptors are stereoselective for the (+) isomers of benzomorphans, and are sensitive to modulation by phenytoin and guanyl nucleotides. In contrast, σ_2 receptors have a lower affinity for (+)-pentazocine, slightly preferring (−) isomers of benzomorphans over (+) isomers, are insensitive to phenytoin and guanyl nucleotide regulation, and may be related to K^+ channel function (Quirion et al., 1992). However, until recently, few physio-

logical functions could be attributed to σ_2 receptor activation. Autoradiographic studies have demonstrated the wide distribution of σ receptors throughout the brain (Gundlach et al., 1986; McLean and Weber, 1988) and subsequent findings about σ receptor function have been reviewed (Walker et al., 1990). We have previously demonstrated that both σ_1 and σ_2 receptors regulate catecholamine release from rat striatum (Gonzalez-Alvear and Werling, 1994), rat cerebellum (Gonzalez-Alvear et al., 1995) and guinea pig nucleus accumbens and prefrontal cortex (Weatherspoon et al., 1996).

Sigma receptors have also been localized to the hippocampus (Mash and Zabetian, 1992), and recent studies have reported physiological effects of σ receptor ligands on hippocampal function (Monnet et al., 1992; Bergeron et al., 1995). Some σ receptor ligands have also been shown to regulate acetylcholine release (Junien et al., 1991) and norepinephrine release (Monnet et al., 1992, 1996; Gonzalez-Alvear and Werling, 1995) in the hippocampus. The compound BD737 binds with K_i values of 1.3 nM at σ_1 receptors (Bowen et al., 1992) and 500 nM at σ_2 receptors (Rothman et al., 1991). (+)-Pentazocine has been reported to have K_i values of 7 nM at σ_1 receptors and 440 nM at σ_2 receptors (Connor and Chavkin, 1992). Using BD737 and (+)-pentazocine as σ receptor agonists, we observed that both σ_1 and σ_2 receptors contribute to regulation of norepinephrine release from rat hippocampal slices (Gonzalez-Alvear and Werling, 1995). Non-subtype selective σ receptor antagonists fully reversed, and a σ_1-selective receptor antagonist, DuP 734, partially reversed inhibition by (+)-pentazocine. Binding studies support the selectivity of DuP 734 as a σ_1 receptor antagonist. [^{3}H]DuP 734 has been shown to label a single site in guinea pig brain, and this binding was displaced by several drugs with dissociation constants that are essentially equivalent to their dissociation constants for competition against [^{3}H]3-PPP binding to σ_1 receptors (Culp et al., 1992; Hellewell and Bowen, 1990). Most notable in discriminating between the two σ sites in both studies were the dissociation constants for (+)-pentazocine and (+)-SKF-10047. In addition, DuP 734 competed for 1 nM [^{3}H]SKF-10047 binding with greater than 90% competition achieved at concentrations below 100 nM (Tam et al., 1992). More recently, when DuP 734 was tested against [^{3}H]DTG binding to σ_2 receptors, it was found to exhibit no competition at concentrations up to 1 μM (Dr. Paul Gilligan, DuPont Merck, personal communication), with higher concentrations not tested. The K_i for the single site labeled, identified as σ_1 receptors, is 10 nM (Culp et al., 1992; Tam et al., 1992). We used a 100 nM concentration of DuP 734 to block binding to σ_1 receptors based on its affinity for these receptors. At this concentration, DuP 734 should produce almost total occupancy (91%) of σ_1 receptors. Thus, at 100 nM, it is likely that DuP 734 exhibits negligible binding to σ_2 receptors.

DuP 734 fully reversed inhibition of stimulated norepinephrine release mediated by BD737 in our earlier experiments (Gonzalez-Alvear and Werling, 1995). Although a significant portion of regulation of NMDA-stimulated norepinephrine release appeared to be via a σ_2 receptor, at that time we had no σ_2-selective drugs with which to confirm this hypothesis. In the current study, we tested σ regulation of norepinephrine release in slices of guinea pig hippocampus, a tissue in which a larger percentage of inhibition appeared to be via σ_2 receptors. We observed that both BD737 (0.1 nM–100 nM) and (+)-pentazocine (500 nM–5 μM) produced inhibition of NMDA-stimulated [^{3}H]norepinephrine release.

To further examine σ_2 regulation of NMDA-stimulated norepinephrine release from hippocampal slices, we employed the σ_2 receptor ligand BIMU-8, whose binding profile had been determined (Bonhaus et al., 1993), but whose agonist/antagonist status at σ_2 receptors had not been identified. We examined whether BIMU-8 might be a σ_2 receptor antagonist. We tested the σ receptor agonists (+)-pentazocine and BD737, both alone and in the presence of Dup 734, or in the presence of BIMU-8. When BIMU-8 was tested alone, it had no effect on basal or NMDA-stimulated [^{3}H]norepinephrine release. BIMU-8 also had no effect on inhibition of stimulated norepinephrine release by BD737 at 50 nM, a concentration chosen to selectively activate σ_1 receptors. However, BIMU-8 significantly, but not completely reversed the inhibition of stimulated release produced by 1 μM (+)-pentazocine. This concentration of (+)-pentazocine should produce nearly complete occupation of σ_1 and σ_2 receptors. We also tested the potential effects of BIMU-8 when the σ_1 component of (+)-pentazocine inhibition was blocked by inclusion of DuP 734. Under these conditions, increasing concentrations of BIMU-8 displayed a concentration-dependent reversal of (+)-pentazocine-mediated inhibition of stimulated release.

We then examined the effect of a 100 nM concentration of BIMU-8 against inhibition produced by a range of concentrations of (+)-pentazocine. When σ_1 receptors were blocked by DuP 734, BIMU-8 fully reversed the remaining inhibition produced by (+)-pentazocine, which at the highest concentration tested would be expected to occupy greater than 90% of σ_2 receptors. These results complement our previous observations in which (+)-pentazocine-mediated inhibition of NMDA-stimulated [^{3}H]norepinephrine release was only partially reversed by DuP 734 but was completely reversed by a combination of DuP 734 and haloperidol (Gonzalez-Alvear and Werling, 1995).

The present results suggest that BIMU-8 acts as an antagonist at σ_2 receptors and that σ_2 receptors contribute to regulation of norepinephrine release in hippocampus. Information regarding the functions of different σ receptor subtypes may be useful in the identification or development of other subtype-selective compounds such as BIMU-1 or BIMU-8 (Bonhaus et al., 1993). Studies to

further understand the physiological roles of σ receptors in the hippocampus will also benefit from the development of such compounds.

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