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IP_3 receptor antagonist heparin uncompetitively inhibits [^{3}H](+)-SKF-10047 binding to σ receptors

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Abstract

Interaction of σ receptors with intracellular Ca^{2+} channel blocker and modulators was examined. Ryanodine and inositol 1,4,5-trisphosphate (IP_3) did not inhibit [^{3}H](+)-*N*-allylnormetazocine ([^{3}H](+)-SKF-10047) binding to σ receptors from either brain microsomal fractions or liver membrane extracts of the rat. However, the IP_3 receptor antagonist heparin inhibited [^{3}H](+)-SKF-10047 to σ receptors in an uncompetitive manner with a K_i of 93 μM. These results suggest that σ receptors may bear some relationship with IP_3 receptor associated proteins or channels.

Keywords: σ Receptor; Ryanodine; Inositol 1,4,5-trisphosphate)

σ Receptors are naloxone-insensitive, widely distributed, [^{3}H](+)-SKF-10047-binding proteins which are different from opioid receptors and/or phencyclidine/*N*-methyl-D-aspartate receptors (Su, 1991). Although the sequence of the σ receptor is unknown at present, recent studies indicate that σ receptors may modulate intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). For example, a selective σ ligand 2-(4-morpholino)ethyl-1-phenylcyclohexane-1-carboxylate hydrochloride (PRE-084) was found to block the learning and memory impairment in mice induced respectively by dizocilpine, mecamylamine, and nimodipine – drugs that are known to reduce $[Ca^{2+}]_i$ (Maurice et al., 1994, 1995). Moreover, in adenocarcinoma cells σ ligands induced a rise in $[Ca^{2+}]_i$ in a medium free of extracellular Ca^{2+} (Brent et al., 1996).

In cells, the $[Ca^{2+}]_i$ is largely regulated by endoplasmic reticulum by two processes: the sequestration of free Ca^{2+} through an energy-dependent process and the release of the stored Ca^{2+} via two Ca^{2+} channels – a ryanodine-sensitive channel and an IP_3-sensitive channel. Ryanodine is a direct channel blocker of the ryanodine-sensitive channel and IP_3 gates the channel via an IP_3 receptor at which heparin, in μM concentration, is a well known antagonist (Ehrlich and Watras, 1988). We speculated that σ receptors might modulate $[Ca^{2+}]_i$ via those two channels by perhaps acting as regulatory proteins on either the ryanodine or the IP_3 receptor. If the speculation is true, a possibility exists that binding of a ligand to its receptor might be affected by ligands of other receptors. This study examined if the blocker (i.e., ryanodine) and modulators (i.e., IP_3 and heparin) of the endoplasmic reticulum Ca^{2+} channels might affect the binding of a prototypic σ receptor ligand [^{3}H](+)-SKF-10047 to σ receptors.

σ Receptors were prepared as previously described from rat brain microsomal fractions and rat liver membrane extracts which are enriched in σ receptors (McCann and Su, 1991). [^{3}H](+)-SKF-10047 (59 Ci/mmol) and [^{3}H]ryanodine (50 Ci/mmol) were from Dupont NEN (Wilmington, DE). Ryanodine was from Research Biochemicals International (Natick, MA). IP_3 and heparin (average molecular weight = 3000; from bovine intestinal mucosa) were from Sigma Chemicals (St. Louis, MO). The molecular weight of heparin was arbitrarily designated as 3000 in this report. Detailed procedures for the binding assay were described elsewhere (McCann and Su, 1991). Briefly, 25 nM of [^{3}H](+)-SKF-10047 and 40 nM of [^{3}H]ryanodine were used in competition assays. Increasing concentrations of [^{3}H](+)-SKF-10047 (5–600 nM) were used in the Scatchard analyses. Nonspecific binding was defined by 10 μM haloperidol or 10 μM ryanodine respectively.

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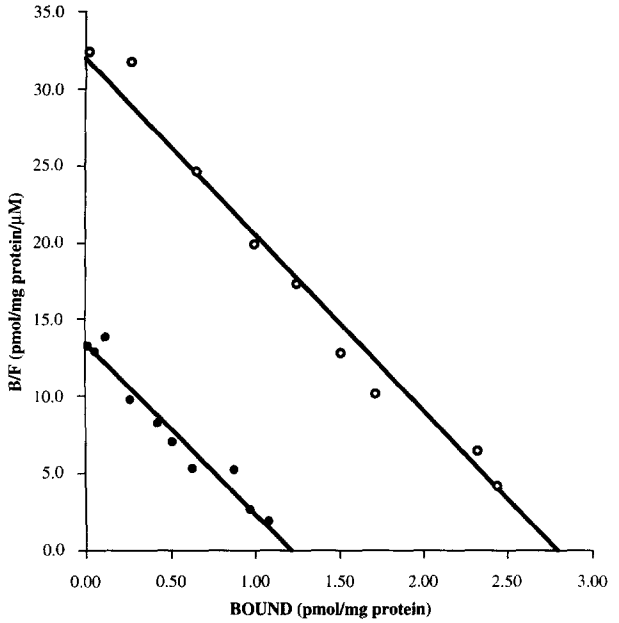

Fig. 1. Scatchard analysis of [^{3}H](+)-SKF-10047 binding to σ receptors: effect of heparin. σ Receptors were prepared from rat liver membrane extracts and incubated with increasing concentrations of [^{3}H](+)-SKF-10047 (5–600 nM) in the absence and presence of 200 μM heparin. The nonspecific binding was defined with 10 μM haloperidol. Data are from a representative experiment which was repeated three times, each assayed in duplicate. ○, control; ●, with 200 μM heparin.

Ryanodine was a poor inhibitor in inhibiting [^{3}H](+)-SKF-10047 binding to σ receptors (less than 50% inhibition at 1.7 mM ($n = 3$) and 7.9 mM ($n = 5$) respectively in assays using brain microsomal fractions and liver membrane extracts). Conversely, (+)-SKF-10047 did not inhibit [^{3}H]ryanodine binding to ryanodine receptors (results not shown). IP_3 was also a poor inhibitor in inhibiting [^{3}H](+)-SKF-10047 binding to σ receptors ($IC_{50} > 3.8$ mM in assays using liver membrane extracts; $n = 2$). However, the IP_3 receptor antagonist heparin at μM concentrations inhibited [^{3}H](+)-SKF-10047 binding to σ receptors ($K_i = 221 \pm 27$ μM ($n = 3$) and 93 ± 11 μM ($n = 4$) respectively using brain microsomal fractions and liver membrane extracts). Heparin inhibited [^{3}H](+)-SKF-10047 binding to σ receptors in an uncompetitive manner. In the presence of 200 μM heparin, the B_{max} of [^{3}H](+)-SKF-10047 binding to σ receptors in the liver membrane extracts was reduced from 2.96 pmol/mg protein (± 0.3 μM; $n = 3$) to 1.24 pmol/mg protein (± 0.1 μM; $n = 3$; Fig. 1). The affinity of [^{3}H](+)-SKF-10047 to σ receptors remained unaltered ($K_i = 90 \pm 8$ nM vs. 92 ± 2 nM).

Our results demonstrating the uncompetitive inhibition of [^{3}H](+)-SKF-10047 binding to σ receptors by heparin suggest that σ receptors might reside in close proximity to IP_3 receptors. In alignment with this speculation are the following observations: (1) both σ receptors and IP_3 receptors are enriched in the microsomal fraction; (2) heparin antagonizes the IP_3 effect and inhibits σ receptor binding both at μM concentration; (3) regional distributions of IP_3 receptors and σ receptors in the brain exhibit similar patterns: highest densities in the molecular layer of cerebellum and CA_1 region of the hippocampus, moderate densities in the striatum and cerebral cortex, and lowest densities in the thalamus, hypothalamus, and substantia gelatinosa (Largent et al., 1986; Worley et al., 1987). Thus, although further studies are required, our results suggest that σ receptors may reside close to IP_3 receptors, thereby affecting $[Ca^{2+}]_i$ in an as yet unknown manner.

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