

**TITLE:** EFFECT OF ISOFLURANE ON GUANINE NUCLEOTIDE INHIBITION OF 5HT<sub>1A</sub> AGONIST BINDING IN FISCHER 344 RAT BRAIN.

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Destabilization of hormone/receptor/G-protein ternary complex by guanine nucleotides was studied in the presence and absence of isoflurane to test the hypothesis that this short term regulation of the initial steps of signal transduction mediated by G-linked receptors is affected by potent inhaled anesthetics. The 5HT<sub>1A</sub> receptor (labeled with 1 nM [<sup>3</sup>H]-8-hydroxy-2-(di-n-propylamino)-tetralin (DPAT)) was chosen as a model. Quantitative receptor autoradiography with 15  $\mu$ m thick coronal sections of Fischer 344 rat brain was studied at the level of the dorsal hippocampus.

As in other rodent models<sup>1</sup>, the highest amount of specific binding was localized to the CA1 region, specifically

the dentate gyrus of the hippocampus. The amount of specific binding appeared to be greater in the presence of 1% isoflurane. In both the presence and absence of anesthetic, the guanine nucleotide GppNHp attenuated 5HT<sub>1A</sub> receptor binding in a concentration dependent manner. The IC<sub>50</sub> for GppNHp inhibition of DPAT binding was approximately 0.1  $\mu$ M and was increased significantly in the presence of isoflurane. These results are qualitatively similar to a different model (muscarinic receptor in rat brain stem) studied by Anthony et. al.<sup>2</sup> The autoradiographic methodology, however, provides a more exacting and biologically less disruptive quantitative assessment of G-protein interaction with anesthetic. This approach should provide further insight into both the nature of and the anatomic localization of the interaction between anesthetic agents, specific receptors, and G-proteins.

#### References

1. Brain Res. 346:205-230,1985
2. Neurosci. Lett. 99:191-196,1989

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**TITLE:** DIFFERENTIAL *C-FOS* ONCOGENE EXPRESSION IN MECHANICALLY-VERSUS CHEMICALLY-INDUCED VISCERAL NOCICEPTION

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The visceral pain model (VPM) based on mechanical distention of the duodenum was used in this study to determine if a mechanically-induced nociceptive stimulus evoked differential *c-fos* expression from a chemically-induced stimulus, i.e. acetic acid writhing (AAW) test. We have suggested that mechanical and chemical visceral pain invoke different nociceptive mechanisms since NSAIDs (acetylsalicylic acid and mefenamic acid) were ineffective in the VPM but were antinociceptive in the AAW test<sup>1</sup>, and there is a differential modulation by spinal opiates in mechanical (VPM) and chemical visceral nociception (in press). The *c-fos* proto-oncogene encodes a nuclear phosphoprotein that is a marker for neuronal activity<sup>2</sup> and has been shown to be differentially expressed following somatic, articular and visceral stimulation<sup>3</sup>. Rats were implanted with balloon catheters in the proximal duodenum 4 days prior to a behavioral testing paradigm. The balloon was inflated to a volume to produce writhing-like activity. Intraperitoneal injection of acetic acid in awake rats was done on another group. One hour later the rats were killed by cardiac perfusion of fixative, the spinal cord removed and 14  $\mu$ m sections of levels T4- L4 were immunostained with a rabbit polyclonal antiserum directed against *c-fos*. In a third group of rats, HRP (horseradish peroxidase) was applied to the denuded section of the proximal duodenum (where the balloon catheter is placed) to determine retrogradely labelled transport to the spinal cord.

Intraperitoneal acetic acid induced *c-fos* like immunoreactivity (FLI) predominately in the superficial dorsal horn (lamina I) and lamina X throughout the levels studied with the densest staining in the thoracolumbar junction. In contrast, the VPM induced intense staining bilaterally in laminae I, II, nucleus proprius, laminae V, VI, and X. The FLI was most dense in those levels which demonstrated HRP (i.e. T6-T9). Using a microcomputer imaging device (MCID), the number of *c-fos*-immunoreactive neurons, using staining area and density thresholds, were quantitated in 3 regions of the right dorsal horn (R1 - lam I, R2 - lam II and III, R3 - lam IV V, and VI). Representative data are shown below (mean  $\pm$  sem) for spinal cord levels T8 and L1 in control, AAW and VPM groups.

		R1	R2	R3
SHAM-CON	(L1)	0.4 $\pm$ 0.3	0.6 $\pm$ 0.3	0.7 $\pm$ 0.5
AAW	(T8)	16.0 $\pm$ 1.9	12.3 $\pm$ 2.6	9.8 $\pm$ 1.4
	(L1)	57.1 $\pm$ 2.4	17.9 $\pm$ 1.6	11.4 $\pm$ 1.8
VPM	(T8)	70.5 $\pm$ 6.0	68.9 $\pm$ 7.9	152.0 $\pm$ 14.4
	(L1)	24.0 $\pm$ 2.3	12.0 $\pm$ 1.3	6.6 $\pm$ 0.7

These data demonstrate that the frequency and distribution of FLI neurons are markedly different for a mechanically-versus a chemically-induced noxious stimulus and supports further evidence for differential spinal processing. Our preliminary data have shown that systemic morphine selectively suppresses FLI in the dorsal horn following duodenal distention. We are presently using *c-fos* expression as a marker to determine what nociceptive neurotransmitters, e.g. peptides, may be causing its induction. These studies may lead to further insight into the clinical treatment and management of chronic visceral pain.

1. DeLeo JA, Colburn RW, Coombs DW, Ellis MA: Pharm. Biochem. & Beh. 33:253-255, 1989.
2. Hunt Sp, Pini A, Evan G: Nature 328:632-634, 1987.
3. Menetrey D, Gannon A, Levine JD, Basbaum A: J. Comp. Neurol. 285:177-195, 1989.