Spinal Analgesic Interaction Between Non-Steroidal Anti-Inflammatory Drugs and *N*-Methyl-D-Aspartate Receptor Systems

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To Fernanda

Abstract

Activation of spinal *N*-methyl-D-aspartate (NMDA) receptors stimulates cyclooxygenase and nitric oxide pathways. Compounds that block the activity of these NMDA receptor systems reduce pain hypersensitivity. However, their usefulness is limited by the side effects they produce. One way of reducing side effects is by combining drugs that produce the same overt effect by different mechanisms, which hopefully increase the net effect. In these series of studies, drugs that interact with NMDA receptor systems and their combinations were screened *in vitro* to identify spinal antinociceptive synergistic combinations that could be assessed *in vivo*. Based on developmental changes in thresholds, conduction velocities and blocking actions of the local anaesthetic lignocaine in neonatal rat L4/L5 dorsal root potentials, it was decided to use spinal cord *in vitro* preparation from 5- to 7-day-old rat pups. In single drug studies, the NMDA receptor channel blocker ketamine (1-50 μM) and the non-steroidal anti-inflammatory drug (NSAID) ketoprofen (200-600 μM), but not the NSAID salicylate (1000 μM) and the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME; 1-100 μM), reduced spinal NMDA receptor-mediated transmission. Ketamine also depressed non-NMDA receptor-mediated transmission. Using isobolographic and composite additive line analyses, fixed-ratio combinations of ketamine and ketoprofen, ketamine and L-NAME, and ketopofen and L-NAME synergistically depressed NMDA receptor-mediated transmission. The two former combinations had a subadditive effect on non-NMDA receptor-mediated transmission, and the latter had no significant effect. These studies identified that all combinations synergistically reduced both nociceptive transmission and potential side effects. In free-moving sheep implanted with indwelling cervical intrathecal catheters, 100 μl subdural administration of ketamine (25-400 μM) and ketoprofen (200-3200 μM) alone and in a fixed-ratio combination (873.95-3350.78 μM, 0.045:0.955) did not raise nociceptive thresholds as assessed by mechanical stimulation of one foreleg. Subdural administration of NMDA (2 mM) decreased mechanical nociceptive thresholds, and this was prevented by the highest concentrations of ketamine and ketoprofen alone and in combination. These findings demonstrated that NMDA receptor channel blockers and NSAIDs alone or in combination had no direct hypoalgesic effects when given onto the spinal cord of sheep, but they prevented NMDA-induced pain hypersensitivity. Simultaneous blockade of NMDA receptor systems could have important clinical implications.

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Abbreviations

8-pCPT-cGMP	8-para-clorophenylthio cGMP, a membrane-permeable cGMP analogue
$[Ca^{2+}]_i$	intracellular calcium concentration
AA	arachidonic acid
$AACOCF_3$	arachydonyl trifluoromethylketone, a type-unspecific PLA_2 inhibitor
aCSF	artificial cerebrospinal fluid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid
AUC	area under the curve
C5	fifth cervical vertebra
CaM	Ca^{2+}-calmodulin complex
cAMP	3',5'-cyclic adenosine monophosphate
CB	cannabinoid
cGMP	3',5'-cyclic guanosine monophosphate
CGRP	calcitonin gen-related peptide
CI	confidence intervals
CNS	central nervous system
COX	cyclo-oxygenases
$cPLA_2$	cytosolic Ca^{2+}-dependent PLA_2
CSF	cerebrospinal fluid
DAG	diacylglycerol
DRCAP	dorsal root compound action potential
DRG	dorsal root ganglion
DR-VRP	dorsal root evoked population ventral root potential
eNOS	endothelial NOS
epsp	excitatory postsynaptic potential
GABA	γ-aminobutyric acid
i.p.	intraperitoneal
i.t.	intrathecal
IC_{40}	inhibitory concentration at 40% depression of maximum possible effect
IC_{50}	inhibitory concentration at 50% depression of maximum effect, median inhibitory concentration
iNOS	inducible NOS
$InsP_3$	inositol-(1,4,5) triphosphate
$iPLA_2$	cytosolic Ca^{2+}-independent PLA_2

L-NAME	N^{ω}-nitro-L-arginine methyl ester
L-PGDS	lipocalin-type PGD synthase
MAFP	methyl arachydonyl fluorophosphanate, a type-unspecific PLA_2 inhibitor
MPE	maximum possible effect
mPGS1	microsomal PGE synthase 1
mPGS2	microsomal PGE synthase 2
MSR	monosynaptic compound action potential
N	Newtons
NF-κB	nuclear factor-κB
NK_1	$neurokinin_1$ receptor
NMDA	*N*-methyl-D-aspartate
nNOS	neuronal NOS
NO	nitric oxide
NOS	nitric oxide synthase
NSAIDs	non-steroidal anti-inflammatory drugs
ODQ	1*H*-[1,2,4]oxadiazolo[4,3-α]qinoxalin-1-one, a gyanylate cyclase blocker
PCOX-1a	partial COX-1a
PCOX-1b	PCOX-1a
PGDS	PGD synthases
PGES	PGE synthase
PGFS	PGF synthase
PGs	prostaglandins
PKA	protein kinase A
PKC	protein kinase C
PKG	cGMP-dependent protein kinases
PLA_2	phospholipase A_2
PS	phosphatidylserine
PSD-93	postsynaptic density-93
PSD-95	postsynaptic density-95
Rp-8-p-CPT-cGMPS	Rp-8-p[(4-Chlorophenyl)thiol]-cGMPS triethylamine, a selective PKG-Iα inhibitor
SP	substance P
$sPLA_2$	secretory PLA_2