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

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Veterinary Science

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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 Nω-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 ketoprofen 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

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<tr>
<td>8-pCPT-cGMP</td>
<td>8-para-clorophenylthio cGMP, a membrane-permeable cGMP analogue</td>
</tr>
<tr>
<td>[Ca^{2+}]_i</td>
<td>intracellular calcium concentration</td>
</tr>
<tr>
<td>AA</td>
<td>arachidonic acid</td>
</tr>
<tr>
<td>AACOCF₃</td>
<td>arachydonyl trifluoromethylketone, a type-unspecific PLA₂ inhibitor</td>
</tr>
<tr>
<td>aCSF</td>
<td>artificial cerebrospinal fluid</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>C5</td>
<td>fifth cervical vertebra</td>
</tr>
<tr>
<td>CaM</td>
<td>Ca^{2+}-calmodulin complex</td>
</tr>
<tr>
<td>cAMP</td>
<td>3',5'-cyclic adenosine monophosphate</td>
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<tr>
<td>CB</td>
<td>cannabinoid</td>
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<td>diacylglycerol</td>
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<td>DRCAP</td>
<td>dorsal root compound action potential</td>
</tr>
<tr>
<td>DRG</td>
<td>dorsal root ganglion</td>
</tr>
<tr>
<td>DR-VRP</td>
<td>dorsal root evoked population ventral root potential</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial NOS</td>
</tr>
<tr>
<td>epsp</td>
<td>excitatory postsynaptic potential</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>i.t.</td>
<td>intrathecal</td>
</tr>
<tr>
<td>IC_{40}</td>
<td>inhibitory concentration at 40% depression of maximum possible effect</td>
</tr>
<tr>
<td>IC_{50}</td>
<td>inhibitory concentration at 50% depression of maximum effect, median inhibitory concentration</td>
</tr>
<tr>
<td>iNOS</td>
<td>inducible NOS</td>
</tr>
<tr>
<td>InsP₃</td>
<td>inositol-(1,4,5) triphosphate</td>
</tr>
<tr>
<td>iPLA₂</td>
<td>cytosolic Ca^{2+}-independent PLA₂</td>
</tr>
</tbody>
</table>
L-NAME \( \Lambda^\text{N}\)-nitro-L-arginine methyl ester
L-PGDS lipocalin-type PGD synthase
MAFP methyl arachidonoyl fluorophosphonate, a type-unspecific PLA\(_2\) inhibitor
MPE maximum possible effect
mPGS\(_1\) microsomal PGE synthase 1
mPGS\(_2\) microsomal PGE synthase 2
MSR monosynaptic compound action potential
N Newtons
NF-kB nuclear factor-kB
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 1H-[1,2,4]oxadiazo[4,3-\(\alpha\)]qinoxalin-1-one, a guanylate 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-1\(\alpha\) inhibitor
SP substance P
sPLA\(_2\) secretory PLA\(_2\)