DA-5018, a Novel Vanilloid Type Analgesic

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Capsaicin (8-methyl-N-vanillyl-6-nonenamide, Fig. 1), a pungent principle present in a wide variety of red peppers of the genus Capsicum, exerts diverse and peculiar biological effects. Since the report of Jancso (1968) that capsaicin application renders man and animals insensitive to further noxious stimuli, capsaicin has been extensively used as an important pharmacological tool in sensory neuroscience. In addition, there have been several attempts to develop a new type of orally active non-steroidal antiinflammatory drugs (NSAIDs) with capsaicin as a lead compound (Lee *et al.*, 1986; Brand *et al.*, 1987). In this approach, systematic modification of the capsaicin structure was executed to optimize the therapeutic index, defined as the ratios of antinociceptive and antiinflammatory potencies to acute toxicity. Among the new compounds, N-[3-(3,4-dimethylphenyl)propyl]-4-(2-aminoethoxy)-3-methoxyphenylacetamide (DA-5018, Fig. 1) synthesized by the Korea Research Institute of Chemical Technology (KRICT) was found to have superiority over capsaicin in many aspects: higher potency, broad therapeutic range, and oral availability (Park *et al.*, 1993). However, unlike other vanilloid-type compounds, phenolic hydroxy group of DA-5018 is 2-aminoethylated. In the previous structure-activity relationship studies, phenolic hydroxy group was considered one of the essential motif to exert vanilloid-type actions (Lee, 1991).

The aim of the present study was to elucidate the mechanism of action of DA-5018 and to compare it with those of capsaicin, resiniferatoxin (ultrapotent capsaicin analog), and olvanil (systemic antinociceptive agent equipotent with capsaicin developed by Proctor & Gamble) (Fig. 1).

Capsaicin causes depletion of various neuropeptides, especially substance P (SP) and calcitonin gene-related peptide (CGRP), from the cell bodies and terminals of nociceptive sensory neurons (Holzer, 1988). To clarify the action mechanism of DA-5018, release of SP and CGRP was quantified by radioimmunoassay. Capsaicin (50 mg/kg), resiniferatoxin (RTX, 0.3 mg/kg), olvanil (200 mg/kg) and DA-5018 (50 mg/kg) reduced the density of SP- and CGRP-like immunoreactivity in the dorsal horn of rat spinal cord, particularly in substantia gelatinosa at 3 weeks of age, and the reduction of immunoreactivity lasted by 10 weeks of age (Table I). This result indicates similarity in action mechanism of capsaicin and DA-5018.

Capsaicin exerts an exciting, desensitizing, and neurotoxic effects on a subset of primary afferent neurons by opening a non-selective cation channels permeable to Na^+, K^+, and especially Ca^{2+} which is essential to induce neuropeptide release and desensitization. ^{45}Ca uptake experiments were conducted

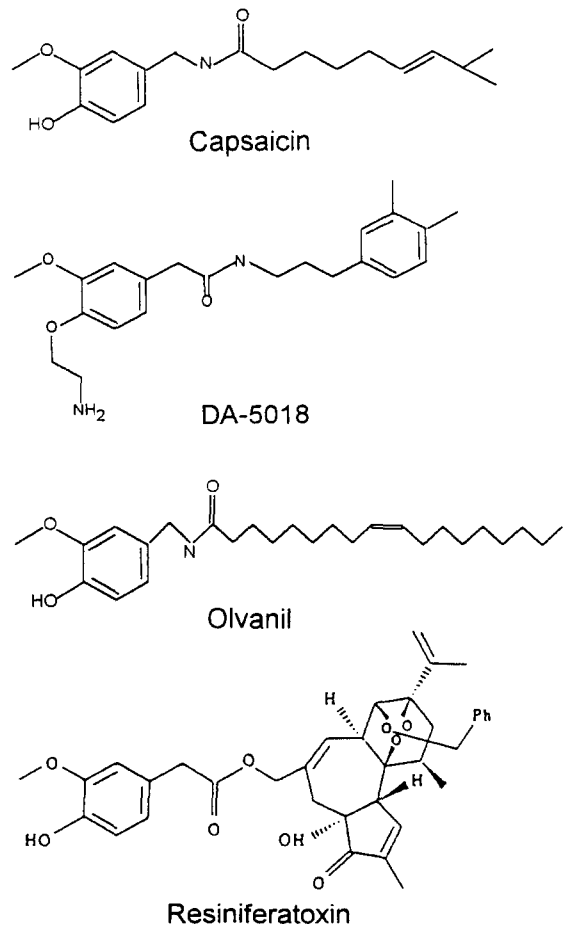

Fig. 1. Structures of capsaicin, DA-5018, olvanil and resiniferatoxin.

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Table I. SP-like immunoreactivity (SP-LI) in the dorsal half of the rat spinal cord as a function of age after neonatal administration of capsaicinoids

Capsaicinoids	Dose (mg/kg)	Substance P (pg/μg protein)					
		3 weeks		7 weeks		10 weeks	
			%		%		%
Vehicle		44.3±0.94	100	39.2±0.35	100	40.3±1.20	100
Capsaicin	50	18.1±1.57	40.9	17.2±5.53	43.9	14.8±0.41	36.7
RTX	0.3	8.78±0.76	19.8	8.69±1.15	22.2	11.6±0.58	28.8
Olvanil	200	18.7±0.80	42.2	16.5±1.93	42.1	19.2±1.06	47.6
DA-5018	50	14.2±0.97	32.1	15.0±2.27	38.3	11.6±0.47	28.8

SP-LI was measured by RIA. Each experiment was carried out in the tissue pooled from 3 animals of 3, 7 and 10 weeks age. Values are expressed in mean±S.E. of the data from 4 replicates.

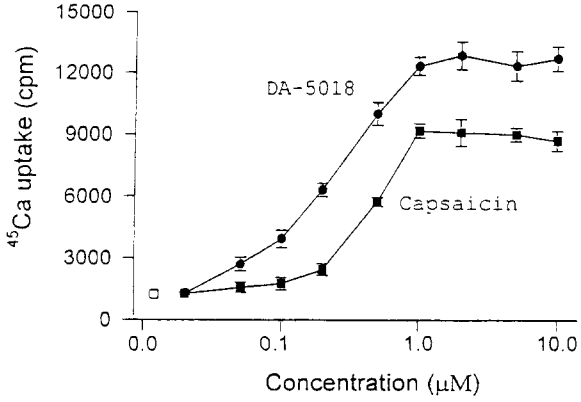

Fig. 2. Dose-response curves for DA-5018 and capsaicin-evoked ^{45}Ca uptake into cultured DRG neurons from neonatal rats. Data are means and SEM from a single representative experiment (n=6). □, background uptake.

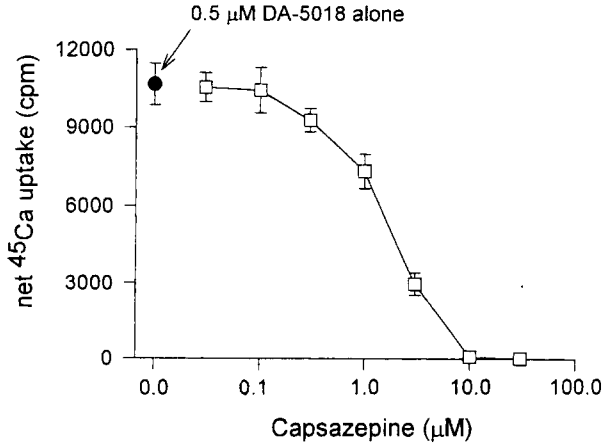

Fig. 3. Inhibition by capsazepine of the uptake of ^{45}Ca evoked by administration of 0.5 μM DA-5018 to neonatal rat cultured DRG neurons. Data are shown as means with SEM indicated by vertical bars, n=6

in cultured DRG neurons prepared from neonatal Sprague-Dawley rats by the method described in detail by Wood *et al.* (1988) with modification. DA-5018 evoked ^{45}Ca uptake into neonatal rat DRG neurons in culture. Dose-response curves for DA-5018- and capsaicin-evoked ^{45}Ca accumulation in a 10 min incubation period are presented in Fig. 2. DA-5018 has not only a lower EC_{50} value (approx. 0.2 μM) than that for capsaicin (0.3 μM), but shows peculiarly higher efficacy. These results confirm the *in vivo* observations of the remarkably potent analgesic action of DA-5018 comparable to that of morphine (Lee *et al.*, 1994). This DA-5018-activated calcium entry into the DRG neurons was subject to dose-dependent antagonism by the capsaicin-specific antagonist, capsazepine (Bevan *et al.*, 1991). When tested against 0.5 μM DA-5018, capsazepine shifted the log dose-response curve to the right (Fig. 3) and, at the concentration of 10 μM, exerted complete abolition of the uptake (IC_{50}≒1 μM). An inhibition study using capsazepine definitely indicated that DA-5018-induced ^{45}Ca uptake is entirely mediated by the vanilloid receptor. Furthermore, voltage-sensitive calcium channel (VSCC) blockers were ineffective to inhibit DA-5018-induced Ca^{2+} uptake. These results verify that the analgesic effect of DA-5018 is mediated by the vanilloid-sensitive neural pathway and by the direct binding to the vanilloid receptor. Whether the difference in potency and efficacy of DA-5018 from capsaicin is attributed to the receptor specificity (Szallasi and Blumberg, 1990), binding affinity or other causes remains to be established.

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