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Distribution of capsaicin in rat tissues after systemic administration

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The pharmacology of capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) was reviewed by Pórszász et al (1955), Szolcsányi (1977) and Virus & Gebhart (1979). Recent interest in capsaicin arose from its effects on the nervous system. Acute effects are (a) stimulation of pain fibres (Jancsó & Jancsó-Gábor 1959; Juan et al 1980), soon followed by desensitisation as shown by impairment of corneal chemosensitivity (Szolcsányi & Jancsó-Gábor 1975), (b) release of substance P (Gamse et al 1979, 1981 a; Theriault et al 1979; Yaksh et al 1980) and somatostatin (Gamse et al 1981 a) from central endings of chemosensitive fibes by a calcium-dependent mechanism and (c) a release of substance P from peripheral endings of these fibres as concluded from indirect evidence (Gamse et al 1981b). Chronic effects of capsaicin on the nervous system are a long-lasting or even irreversible depletion of substance P (Jessell et al 1978; Gamse et al 1980a; Nagy et al 1980; Lembeck & Donnerer 1981) and somatostatin (Gamse et al 1980b) exclusively from a certain type of primary afferent neuron but not from substance P and somatostatin neurons intrinsic to brain. This difference might be due either to an insufficient permeation of the blood brain barrier by capsaicin or to a selective capsaicin sensitivity of those neurons that are depleted by capsaicin. A recently developed method to determine capsaicin in animal tissue (Saria et al 1981) was used to investigate the distribution of capsaicin under experimental conditions which have been applied to achieve depletion of substance P and somatostatin. It was, however, not intended to investigate the entire pharmacokinetic properties of capsaicin.

Methods and materials

Administration of capsaicin. For intravenous injection Sprague Dawley rats of either sex, 200–250 g, were anaesthetized with 35 mg kg^{-1} pentobarbitone. The trachea and the carotid artery were cannulated. Capsaicin, 2 mg kg^{-1}, was injected into the femoral vein and animals were artificially ventilated. Stock solutions contained 20 mg ml^{-1} capsaicin in 0·9% NaCl with 10% ethanol and 10% Tween 80. Appropriate dilutions for i.v. injection were made in 0·9% NaCl. Subcutaneous injections under the back skin (50 mg kg^{-1} capsaicin, i.e. 0·5–0·6 ml stock solution) were performed under light pentobarbitone anaesthesia (25 mg kg^{-1}).

Extraction of tissues and determination of capsaicin. Animals were killed by bleeding from the carotid artery, blood was collected and the following tissues were prepared immediately: whole brain, spinal cord, liver, kidney and omentum. Tissues were weighed and placed in about 10 vol ice cold acetone. After homogenization in glass tubes with a Teflon pestle the homogenates were centrifuged for 10 min at 3000 *g*. The supernatants were evaporated in a Brinkman sample concentrator and redissolved in 2·0 ml methanol. Capsaicin was determined by reversed phase h.p.l.c. as described elsewhere (Saria et al 1981). The recovery of capsaicin from tissues was approximately 90%, the detection limit of h.p.l.c. analysis about 3 ng capsaicin (Saria et al 1981). Capsaicin: E. Merck, A.G., Darmstadt, Germany.

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Results

Intravenous administration of capsaicin. Capsaicin levels in tissues 3 and 10 min after i.v. injection of 2 mg kg^{-1} are shown in Table 1. Three minutes after i.v. administration an approximately 5-fold higher concentration of capsaicin was found in brain and spinal cord and about 3-fold higher value was found in the liver than in blood. Ten minutes after administration, the concentration of capsaicin was greatly decreased in blood and liver. At this time the capsaicin level in spinal cord and also in brain were still higher than that in blood and liver.

Subcutaneous administration of capsaicin. After a single subcutaneous injection of 50 mg kg^{-1} capsaicin, the concentration in blood seemed to follow a Bateman function with a maximum at 5 h. Capsaicin concentrations in brain and spinal cord were nearly as high as the concentrations in blood up to 100 min, thereafter they were slightly lower. The highest capsaicin levels were found in the kidney, whereas the concentration in the liver always remained very low. Capsaicin was already detectable 10 min after s.c. administration in all tissues investigated except adipose tissue (omentum) where measurable amounts were not found earlier than 30 min after application. After 17 h, capsaicin concentrations fell below the detection limit in all tissues except in blood (Fig. 1).

Table 1. Capsaicin in brain, spinal cord, liver and blood 3 and 10 min after i.v. administration of 2 mg kg^{-1}.

Tissue	Capsaicin (ng g^{-1}) 3 min	10 min
Brain	2763 ± 412*	364 ± 88
Spinal cord	2670 ± 280*	916 ± 192*
Liver	1736 ± 355*	46 ± 14
Blood	581 ± 230	48 ± 18

Mean ± s.e.m., N = 6. Values were compared by one way analysis of variance, followed by Duncan's multiple range test.
* $P < 0.05$ vs blood.

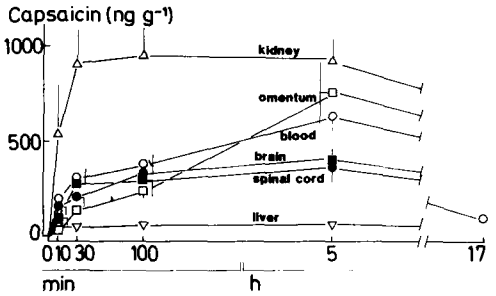

FIG. 1. Subcutaneous administration of 50 mg kg^{-1} capsaicin. Ordinate: Capsaicin concentration in tissue as indicated. Abscissa: Time after injection. $\bar{x} \pm$ s.e.m. N = 6-17 h after administration detectable amounts of capsaicin were found only in the blood.

Discussion

The rapid increase of capsaicin in various organs after s.c. administration is in agreement with the results of Glinsukon et al (1980), who described toxic symptoms occurring a few minutes after s.c. application of capsaicin. Tissue concentrations remained near maximal levels over several hours, suggesting a slow diffusion from the site of injection. The low levels found in the liver are likely the result of a formation of conjugates with glucuronic acid or sulphuric acid. This inactivation step was shown to be the main elimination pathway for ethamivan, a chemically similar compound with a hydroxy-methoxyphenyl group (Beubler 1971). The h.p.l.c. method used detected capsaicin, but not conjugates or metabolites. More polar conjugates would have been eluted within the large front peak and could, therefore, not be identified. Intravenous administration of 1/25 of the s.c. dose led initially to higher tissue levels of capsaicin in brain and spinal cord than after s.c. administration. This indicates a rapid entrance of capsaicin into the central nervous system (c.n.s.). The decrease of tissue levels during the following minutes very likely reflects redistribution into adipose tissue and/or inactivation by formation of conjugates. The lower LD50 value after i.v. administration (1/20 of the LD50 value after s.c. administration, Glinsukon et al 1980) results most probably from much higher c.n.s. tissue concentrations achieved by i.v. injection.

The hydrophobic capsaicin molecule was found to pass the blood brain barrier very easily, resulting in tissue concentrations in the c.n.s. which were close to the concentrations in blood and adipose tissue after s.c. administration. This clearly demonstrates that high concentrations of capsaicin also reach neurons intrinsic to the c.n.s. In contrast, capsaicin has been reported not to deplete substance P or somatostatin from intrinsic c.n.s. neurons, but only from primary sensory neurons. (Gamse et al 1980a.b; Nagy et al 1980; Cuello et al 1981). This selective effect of capsaicin on primary sensory neurons cannot therefore be attributed to selective tissue distribution, but must result from differences in the sensitivity of the two systems of peptidergic neurons. In addition, the present results substantiate indirect evidence for access of capsaicin to c.n.s. structures like the preoptic area which was shown to contain capsaicin sensitive thermoregulatory neurons (Nakayama et al 1978). Szolcsányi et al (1971) described a swelling of perikaryal mitochondria of certain neurons in the preoptic area after s.c. administration of 15–35 mg kg^{-1} capsaicin to rats, accompanied by an impairment of thermoregulation (Jancsó-Gábor et al 1970; Szolcsányi & Jancsó-Gábor 1973; Szolcsányi & Jancsó-Gábor 1974).

The rapid distribution of capsaicin agrees with findings of Lembeck & Donnerer (1981), that abolition of the chemosensitivity of the cornea and inhibition of neurogenic plasma extravasation was observed 10 min after s.c. administration of 50 mg kg^{-1} capsaicin. The long duration (weeks to months) of depletion of substance P and somatostatin in spinal cord and peripheral nerves and the impairment of thermoregulation (cf. refs. quoted), however, cannot be explained by the continuous presence of large amounts of capsaicin in nervous tissues but must be the result of biochemical changes induced by the initially high capsaicin concentrations.

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The effect of electroconvulsive shock at a clinically equivalent schedule on rat cortical β-adrenoceptors

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Vetulani & Sulser (1975) reported that daily electroconvulsive shock (ECS) for seven days reduced rat forebrain noradrenaline-sensitive cyclic (c)AMP accumulation. Bergstrom & Kellar (1979) similarly found that daily ECS for seven days reduced rat cortical binding of the β-blocking compound dihydroalprenol (DHA) by 25%. Pandey et al (1979) found identical results, using daily ECS for 12 days they caused a 27% reduction in rat cortical DHA binding.

It has been proposed that all antidepressant treatments reduce β-adrenoceptor sensitivity (Vetulani & Sulser 1975). However, Lerer et al (1981) noted that some neurochemical effects of ECS disappear when the schedule of treatments is reduced to 3 times per week for a total of 12 treatments. Since in man treatment occurs at a schedule of 3 ECS per week for 9–12 treatments, biochemical effects that can be shown at 3 treatments per week for 9–12 treatments are more likely candidates as biochemical correlates of the therapeutic mechanism of electroconvulsive therapy (ECT). We therefore decided to study the effect of three ECS weekly for 4 weeks on rat cortical β-adrenoceptors.

Male albino rats, sabra strain, 150–200 g were housed four to a cage in identical plastic cages in a temperature-controlled (24 °C) environment with a regular 12-h light-dark cycle, and free access to water. ECS was administered (150V for 1·5 s) through ear clip electrodes, 3 times a week during a four week period. Control animals were identically handled and ear-clipped without current being applied. No anaesthetic was used.

Four days after the last ECS, animals were decapitated and the cortex dissected for assay. The assay was performed after the method of Bylund & Snyder (1976). The weighed frontal cortices were homogenized in 30 volumes of 50 mM Tris-HCl buffer (pH 8·0) centrifuged at 49 000 *g* for 15 min and washed twice with the Tris buffer to obtain a crude membrane preparation. The washed membranes were resuspended in 100 volumes of the Tris buffer and aliquots of 1·0 ml were incubated for 20 min at 25 °C with 0·05 ml aliquots containing varying concentrations of [^{3}H]dihydroalprenolol ([^{3}H]DHA), specific activity 49 Ci mM^{-1}, NEN. Five concentrations of [^{3}H]DHA were used from 2·0 to 12 nM. Binding was terminated by filtration through Whatman GF/B filters. Each Scatchard plot was done on an individual rat cortex.

Results are shown in Table 1. A significant 21% reduction in DHA binding (B_{max}) was observed in the ECS group, with no change in Kd. The 21% reduction found in this experiment appears almost identical to the 27% reported by Pandey et al (1979) or the 25% reported by Bergstrom & Kellar (1979). The latter two groups measured DHA binding one day after the last of a series of ECS. The present results suggest that a reduction of β-adrenoceptor number occurs even after clinically equivalent treatment schedules, and is still present at least four days after the last of 12 ECS. This would support the concept of reduction in β-adrenoceptor number as a biochemical mechanism of ECS.

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Table 1. The Effect of ECS on DHA Binding (*p* mol g^{-1} weight).

	B_{max}	Kd	n
Control	24·5* (s.d. 4·5)	5·5	13
ECS	19·5 (s.d. 4·5)	5·6	12

* $P < 0.05$ (Student's *t*-test, two-tailed).

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