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Effects of neonatal capsaicin administration on the nociceptive response of the rat to mechanical and chemical stimuli

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Acute capsaicin treatment in adult rats has been shown to increase firing rates in peripheral sensory nerves involved in the transmission of nociceptive input due to chemical irritants or thermal stimuli, whereas the firing rate of those fibres involved in the transmission of impulses caused by light touch or mechanical stimuli is unaltered (Coleridge et al 1964). Treatment of rats with capsaicin on day 2 of life induces a selective degeneration of primary sensory neurons involved in the mediation of chemically-induced pain (Jancso et al 1977) and it has been reported that reaction times to thermal stimuli in hot-plate and tail-flick tests measured in these animals 3 months later are prolonged (Holzer et al 1979). Responses to mechanical stimuli remain unchanged (Jancso 1968).

The aim of the present study was to determine the effects of capsaicin treatment in neonatal rats on the nociceptive responses due to chemical and mechanical stimuli using two different test systems. The injection of irritant chemical substances into a lateral tail vein in the rat produces behavioural responses, the severity of which can be graded in a semi-quantitative manner using an arbitary 0–3 scale, where 0 represents no pain as indicated by the lack of any struggling or vocalization; 1 represents slight pain indicated by struggling; 2 represents moderate pain indicated by struggling and the rat turns in an attempt to bite the injecting needle; 3 represents severe pain as indicated by intense struggling and pulling away from the injection. In this latter phase, the rat also vocalizes. In this test, potassium chloride (10^{-1} M), hydrochloric acid (10^{-1} M) and bradykinin (10^{-5} M) produce marked behavioural effects. Hydrochloric acid was selected due to the reproducibility of responses with this agent. Pain were also determined in normal and yeast-inflamed rat paws using a Ugo Basile 'analgesy-meter', which is described by Swingle et al (1971).

On the second day of life, female rats (Alderley Park strain) were dosed with capsaicin (Sigma) 50 mg kg^{-1} or vehicle (0·5% cremophor EL; 2·0% DMSO in 0·9% saline) subcutaneously. Three months later, 0·05 ml of 10^{-1} M hydrochloric acid was injected into the lateral tain vein over a period of 20·0 s and an assessment was made of the behavioural response during the injection. Using the 'analgesy-meter', the pressure on the paw was increased at a rate of 16 g s^{-1} and respectively 16 × 2, 16 × 3, etc. according to the number of additional weights added, responses generally being obtained within 5–10 s, i.e. withdrawal of the paw by the rat. All experiments were performed using the same animals for each test with a one-week interval between the tests when pain thresholds were determined in rats with normal and inflamed paws. The operator was unaware of the treatment administered to each rat.

In the tail-vein injection test, capsaicin treatment resulted in an 81% decrease in the mean pain response (capsaicin-treated 0·4 ± 0·1 n = 7, vehicle-treated 2·0 ± 0·3 n = 7 $P < 0.01$ Student's unpaired t-test). In the rat paw-pressure test, capsaicin treatment resulted in a 63 and 72% increase in nociceptive threshold in rats with normal and inflamed paws respectively (Table 1).

These observations show that it is possible to detect differences in nociceptive thresholds between untreated and treated rats when subjected to both mechanical and chemical stimuli. This former result is at variance

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Table 1. Effects of capsaicin treatment (50 mg kg^{-1} s.c. on day 2 of life) on nociceptive threshold in rats with normal and yeast-inflamed paws in the paw-pressure test 3 months later. Number of animals in parentheses.

	Nociceptive threshold (*g* force) Normal paw	Inflamed paw
Capsaicin treated	570 ± 104 (7)*	443 ± 26 (6)**
Vehicle treated	352 ± 42 (7)	122 ± 24 (7)

* Significantly different to vehicle $P < 0.05$.
** Significantly different to vehicle $P < 0.001$ using Student's unpaired *t*-test.

with the findings of Jansco et al (1977). The reasons for this difference could be that the previous workers failed to accurately quantify the response to a mechanical stimulus and that the stimulus might have been of a different quality, i.e. a pinch rather than a gradual increase in pressure. Moreover, in the rat paw-pressure test it could be that as pressure to the paw is gradually increased, release of a chemical pain mediator may occur, leading to activation of chemoreceptors in addition to the already activated mechanoreceptors. In fact, it has been demonstrated that capsaicin treatment reduces the size of antidromically evoked action potentials in C_2-type fibres, having no effect on C_1, A-α-β and A-δ-type fibres (Szolcsanyi 1977). Our results confirm that capsaicin treatment is more effective in a situation where the main influence on the pain response is chemical and possibly via C-type fibres, i.e. when the paw is inflamed or a noxious substance is injected intravenously. In the case of the normal paw it could be that the mechanical stimulation leads to activation of the A-δ-type fibres and that these are subserved by the C-type fibres that are affected by capsaicin treatment.

In interpreting the result obtained in the yeast-inflamed paw, the possibility that capsaicin may also have reduced the severity of the yeast-induced inflammatory response must be considered. Indeed, it has been demonstrated that rats treated with capsaicin on day 2 of life and used 4 months later, show significantly reduced hind-paw vasodilation following antidromic stimulation (Lembeck & Holzer 1979). Further, Gamse et al (1980) were able to inhibit neurogenic plasma extravasation by more than 80% in rats treated in an identical fashion. Thus, it is highly probable that capsaicin could have reduced the inflammatory response in our experiments, and therefore contributed to the more marked analgesic effect observed in these animals.

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The use of Sephadex LH-20 to improve yield in the initial stages of the purification of slow reacting substance of anaphylaxis

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In any investigation of the properties of slow reacting substance of anaphylaxis (SRS-A) it is necessary to purify SRS-A from biological fluids which usually contain inorganic salts, histamine and other pharmacologically active compounds. Many multistep techniques have been reported in which an early step is to separate SRS-A from histamine and physiological salts using either charcoal (Morris et al 1978) or the Amberlite XAD-2, 7 and 8 ion-exchange resins originally described by Orange et al (1973). However, other workers in this field (Takahashi et al 1976; Bach et al 1979; Whelan 1980) failed to obtain high, reproducible yields of SRS-A using such methods. Furthermore in a recent study using Amberlite XAD-8, Lee et al (1979) could only separate SRS-A from histamine by introducing an additional elution into the procedure. Recently, SRS-A was separated from histamine and physiological salts by solvent extraction (Whelan 1980); this method gave a mean recovery of SRS-A of 55 ± 11% which was greater than that obtained using Amberlite XAD-2 chromatography. Therefore, it is desirable that any extraction procedure should be simple and give a reproducible and high percentage yield of SRS-A.

Orange et al (1973) used a column of Sephadex LH-20 to determine the molecular weight of SRS-A and recently Yecies et al (1979) have used Sephadex LH-20 chromatography in the isolation of a slow reacting substance released by the ionophore A 23187. In the experiments described below a calibrated column of Sephadex LH-20 has been used to separate SRS-A from histamine and physiological salts such that over 90% recovery of SRS-A is effected.

Sephadex LH-20 (Pharmacia) was slurried in methanol–water (4 : 1 v/v) and packed into a borosilicate glass