J. Pharm. Pharmacol. 1981, 33: 183–185
Communicated November 17, 1980

0022–3573/81/030183–03 $02.50/0

Effects of neonatal administration of capsaicin on nociceptive thresholds in the mouse and rat

A. G. HAYES, J. W. SCADDING†, M. SKINGLE, M. B. TYERS*, *Pharmacology Department, Glaxo Group Research Ltd., Greenford Road, Greenford, Middlesex, UB6 0HE, and † MRC Cerebral Functions Group, Department of Anatomy, University College, London WC1 6BT, U.K.*

Substance P (SP) is present in unmyelinated primary afferent fibres entering the spinal cord (Hökfelt et al 1975) and has been suggested to be involved in pain transmission in the dorsal horn (Henry 1976; Randić & Miletić 1977). Treatment of 2-day old rats with capsaicin, the active principle of *Capsicum frutescens* (Mexican chilli pepper), results in a degeneration of unmyelinated fibres from the dorsal roots and dorsal horn of the spinal cord (Jancsó et al 1977; Lawson & Nickels 1980; Scadding 1980). These changes also result in a loss of SP from the dorsal horn (Gamse et al 1980; Nagy et al 1980). Janscó et al (1977) reported that such neonatally treated rats did not respond to corneal application of irritant chemicals, but appeared to respond normally to noxious mechanical stimulation (e.g. forceps pinch). Furthermore, Holzer et al (1979) and Nagy et al (1980) have shown slightly increased nociceptive heat thresholds in the tail-flick and hot-plate tests in rats treated neonatally with capsaicin.

In adult rats, repeated doses of capsaicin cause a loss of SP from the dorsal horn of the spinal cord (Jessell et al 1978; Hayes & Tyers 1980), but there is no nerve fibre degeneration. In rats treated with this latter dosing regimen, nociceptive pressure and chemical thresholds are markedly raised for a period of 2–3 months after dosing, but there is no significant increase in nociceptive heat thresholds. Indeed, reaction latencies in the tail-immersion test are slightly reduced (Hayes & Tyers 1980).

The purpose of the present experiments was to determine whether, under our experimental conditions, there was any difference between the antinociceptive effects of capsaicin when given repeatedly to adults and when given to neonates. Thus, the effects of capsaicin, administered to neonate rats and mice, on nociceptive responses evoked by heat, pressure and chemical stimuli have been determined. Any other overt behavioural effects induced by this treatment were also studied.

Thirty male Wistar rats were injected subcutaneously on day 2 of life with capsaicin (Sigma Chemicals Ltd) 50 mg kg^{-1}. Control rats received only the solvent (10% ethanol, 10% Tween 80 in 0·9% NaCl). Twenty-eight female CBA mice were injected similarly on days 2 and 5 of life.

Nociceptive thresholds were determined in the rats when they were 2 months old and in the mice when 5 months old. The animals were randomized between cages such that each cage contained animals of each treatment. Animals were colour-coded according to treatment so that the operator was unaware of which treatments the animals had received.

Three antinociceptive tests were carried out in the rat: the paw pressure test, the hot-plate test and the tail-immersion test. In the paw pressure test, nociceptive pressure thresholds were determined for both hind paws using an 'Analgesymeter' (Ugo Basile, Milan). The nociceptive response was taken as a shrill vocalization or a strong attempt to withdraw the paw. The pressure applied at this point could be read directly from the calibrated scale on the analgesymeter. In the hot-plate test, reaction times of rats placed on a copper plate heated to 55 ± 0·2 °C were determined. A front paw lick was taken as the nociceptive response, at which time the rat was rapidly removed from the hot-plate; 60 s was taken as the maximum reaction time. In the tail-immersion test the rat's tail was placed in hot water at 50 ± 0·2 °C and the times taken for the rats to flick their tails or withdraw them from the water were determined.

Two antinociceptive tests were carried out in the mouse: the hot-plate (55 °C) test, which was carried out as described for the rat, and the acetylcholine-induced abdominal constriction test. In this latter test, mice were injected with acetylcholine, 3 mg kg^{-1} intraperitoneally. The nociceptive response produced was a contraction of the abdominal muscles accompanied by an extension of the hind limbs. The number of responses occurring during the first 5 min after injection was recorded.

To detect any adverse behavioural or incapacitating effects of capsaicin treatment, both the rats and the mice were subjected to a visual observation test, using a method based on that described by Irwin (1968). Changes in various categories of behaviour, including depression, stimulation, reflex activity, muscle tone and autonomic activity, were scored on an arbitrary scale. The animals were also tested for motor incapacitation using an accelerating rotarod (Jones & Roberts 1968), in which the time taken for the animals to fall from the rod was measured. Body weight was recorded only in the mice.

In all tests, any differences obtained between capsaicin treated and control animals were analysed for statistical significance using Student's *t*-test.

* Correspondence.

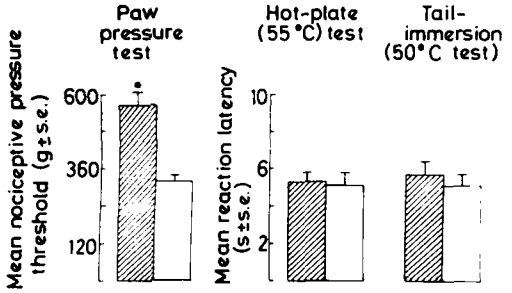

FIG. 1. The effect of neonatal administration of capsaicin on nociceptive pressure and heat thresholds in the rat, measured when the animals were 2 months old. The hatched columns represent capsaicin-treated rats and the open columns vehicle-treated rats.
* represents an effect significantly different from control $P < 0.001$.

The results obtained for the nociceptive thresholds in the rat are shown in Fig. 1. The nociceptive pressure thresholds obtained in the paw pressure test were significantly higher in the rats treated with capsaicin than in the controls. In marked contrast, reaction latencies in both heat tests were not significantly different in capsaicin-treated and control rats.

The results obtained in the nociceptive tests in the mouse are shown in Fig. 2. In the acetylcholine-induced abdominal constriction test, mice treated with capsaicin showed a significantly lower number of abdominal constrictions than controls. However, as in the rat, there was no significant effect on hot-plate reaction latencies.

The results for the behavioural tests are shown in Table 1. In the visual observation test, there were no detectable differences in behaviour produced by capsaicin and vehicle treatments in either rats or mice. Similarly, in the rotarod test, there were no significant differences in reaction latencies between capsaicin-treated and control animals. In the mouse, there was no significant difference in body weight between the 2 groups. These results indicate that neonatal capsaicin treatment produced no adverse behavioural or incapacitating effects which might interfere with the anti-nociceptive testing.

Table 1. The effect of neonatal administration of capsaicin on various behavioural measures in the rat and mouse. For the rotarod incapacitation and body weight measures mean value ± standard errors are shown.

Behavioural measure	Mouse		Rat	
	Capsaicin	Vehicle	Capsaicin	Vehicle
Visual observation	No detectable differences		No detectable differences	
Rotarod incapacitation (latency in s)	56·4 ± 6·3	55·8 ± 9·9	34·7 ± 4·3	47·1 ± 8·1
Body weight in g	19·6 ± 0·7	20·3 ± 0·3	—	—

The results of the antinociceptive tests in the rat and mouse indicate that neonatal treatment with capsaicin produces raised nociceptive pressure and chemical thresholds, but no change in nociceptive heat thresholds. This profile of activity agrees with our previous results obtained with adult rats treated either repeatedly with capsaicin (Hayes & Tyers 1980) or with a single subcutaneous dose of capsaicin (Hayes et al 1980). Jancsó et al (1977) reported that neonatal administration of capsaicin had no effect on responses to noxious mechanical stimulation. However, it is most likely that the intense stimulation produced by forceps pinch is much above the threshold levels measured in the present study and therefore changes in threshold would be more difficult to detect. The apparently conflicting reports on the effects of capsaicin on nociceptive heat thresholds are more difficult to explain. Obal et al (1979) also found that capsaicin produced no defect in the response to heat pain. And, while Holzer et al (1979) and Nagy et al (1980) did show increases in reaction latencies in nociceptive heat tests, the changes were so small that the latter authors concluded that 'capsaicin sensitive neurones must play a relatively minor role in transmission of thermal noxious stimuli'.

The ability of capsaicin to discriminate heat from non-heat nociceptive stimuli has important implications in the neurophysiology of pain. The results obtained in the present study suggest that the primary afferent fibres which are irreversibly damaged by neonatal administration of capsaicin are involved in transmission of noxious pressure and chemical stimuli, but that noxious heat impulses employ a different pathway. This finding is rather difficult to explain in terms of the postulated neurophysiology of cutaneous nociceptive input. Pain impulses are conveyed by unmyelinated 'C' fibres and also by small myelinated

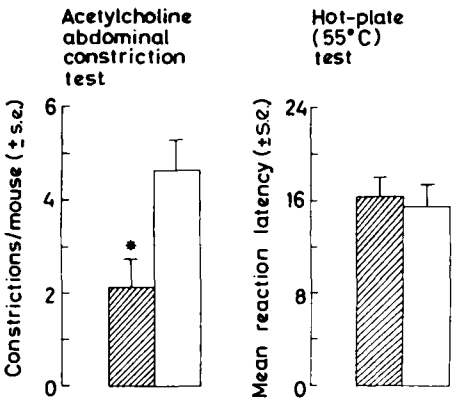

FIG. 2. The effect of neonatal administration of capsaicin on nociceptive chemical and heat thresholds in the mouse, measured when the animals were 5 months old. The hatched columns represent capsaicin-treated mice and the open columns vehicle-treated mice.
* represents an effect significantly different from control $P < 0.05$.

'Aδ' fibres. The 'C' fibre nociceptors consist of the polymodal nociceptors, which respond to nociceptive heat, chemical and mechanical stimuli; and mechanical nociceptors, situated in subcutaneous tissue, which respond only to nociceptive mechanical stimuli. The 'Aδ' fibre nociceptors are mainly specific mechanical nociceptors (Burgess & Perl 1973; Iggo 1974). Neonatal capsaicin administration produces a large decrease in the number of 'C' fibres and has little effect on the 'Aδ' population. This may explain why the forceps pinch response, which is probably 'Aδ' mediated, is not affected by capsaicin; whereas the nociceptive response produced by the blunt point used in our experiments, which is probably 'C' fibre mediated, is altered. It is difficult to see why nociceptive heat thresholds are unchanged, as there must be a large decrease in the number of polymodal nociceptors. It is possible that the rat adapts to use the small number of remaining 'C' fibres to transmit nociceptive heat impulses, whereas input in a large number of fibres is necessary for transmission of nociceptive pressure and chemical impulses.

The fact that dorsal horn SP is depleted by neonatal capsaicin treatment (Gamse et al 1980; Nagy et al 1980) indicates that SP may mediate transmission of pressure and chemically-induced nociception at primary afferent terminals. However, the fact that heat nociception is apparently unaffected by such treatment indicates either that SP is not involved in transmission of nociceptive heat impulses or that there is a high safety factor for SP mediated transmission in this pathway.

The authors would like to thank Mrs Janine Thomson, Mrs Deborah Murray and Dr Alma Gower for technical assistance.

REFERENCES

Burgess, P. R., Perl, E. R. (1973) in: Iggo, A. (ed.) Handbook of Sensory Physiology, Vol. II, The Somatosensory System. Springer-Verlag, Berlin, Heidelberg, New York, pp 29–78

Gamse, R., Holzer, P., Lembeck, F. (1980) Br. J. Pharmacol. 68: 207–213

Hayes, A. G., Tyers, M. B. (1980) Brain Res. 189: 561–564

Hayes, A. G., Skingle, M., Tyers, M. B. (1980) Br. J. Pharmacol. 70: 96P

Henry, J. L. (1976) Brain Res. 114: 439–451

Hökfelt, T., Kellerth, J-O., Nilsson, G., Pernow, B. (1975) Ibid. 100: 235–252

Holzer, P., Jurna, I., Gamse, R., Lembeck, F. (1979) Eur. J. Pharmacol. 58: 511–514

Iggo, A. (1974) in: Bonica, J. J., Procacci, P., Pagni, C. A. (eds) Recent Advances in Pain: Pathophysiology and Clinical Aspects. C. C. Thomas, Springfield USA, pp 3–35

Irwin, S. (1968) Psychopharmacologia (Berlin) 13: 222–257

Jancsó, G., Kiraly, E., Jancsó-Gabór, A. (1977) Nature (London) 270: 741–743

Jessell, T. M., Iversen, L. L., Cuello, A. C. (1978) Brain Res. 152: 183–188

Jones, B. J., Roberts, D. J. (1968) J. Pharm. Pharmacol. 20: 302–304

Lawson, S. N., Nickels, S. M. (1980) J. Physiol. (London) 303: 19P

Nagy, J. I., Vincent, S. R., Staines, W. A., Fibiger, H. A., Reisine, T. D., Yamamura, H. I. (1980) Brain Res. 186: 435–444

Obal, F., Benedek, G., Jancsó-Gabór, A., Obal, F. (1979) Pflügers Arch. 382: 249–254

Randić, M., Miletić, V. (1977) Brain Res. 128: 164–169

Scadding, J. W. (1980) J. Anatomy 131: 473–484

J. Pharm. Pharmacol. 1980, 33: 185–187
Communicated July 1, 1980

0022–3573/81/030185–03 $02.50/0

Relationship between camazepam, *N*-methyl-oxazepam and oxazepam brain concentrations and antileptazol effect in the rat

S. Caccia*, M. Ballabio, S. Garattini, *Instituto di Ricerche Farmacologiche "Mario Negri" Via Eritrea, 62–20157 Milan, Italy*

Camazepam (7-chloro-3,*NN*-dimethylcarbamoyloxy-5-phenyl-1-methyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one) has the pharmacological profile of an anxiolytic agent with a clear separation between anxiolytic action and sedative-depressive side effects (Ferrini et al 1974).

Studies of the metabolism of camazepam indicate that the compound is excreted in the urine of several animal species, partly free and partly as *N*-methyl-oxazepam and oxazepam glucuronides (Garattini et al 1977). *N*-Methyl-oxazepam (Temazepam) and oxazepam are pharmacologically active metabolites (Marcucci et al 1968, 1972; Randall & Kappell 1973; Garattini et al 1973) and may be considered possible contributors to the central nervous system activity of the parent compound.

This hypothesis was investigated in the present experiments by comparing brain concentrations of *N*-methyl-oxazepam and oxazepam after administration of the two metabolites or of camazepam at doses effective against leptazol (pentetrazol) induced convulsions.

Male CD-Sprague Dawley rats (Charles River, Italy) 200 g were used. Benzodiazepines were administered orally, suspended in 0·5% carboxymethylcellulose, or injected intravenously dissolved in propylglycol-glycofurol-benzyl alcohol-water (30:30:2:48) at doses

* Correspondence.