Pharmacological Characterization of the Vanilloid Receptor in the Rat Isolated Vas Deferens

KAY A. WARDLE, GENEVIVE FUREY* AND GARETH J. SANGER

Department of Neurology Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, Essex, UK

Abstract

The present study set out to further characterize the vanilloid receptor in the rat isolated vas deferens.

In this preparation, both capsaicin and resiniferatoxin (RTX) evoked a concentration–dependent inhibition in the amplitude of electrically-evoked contractions with pEC50 values of 7.62 ± 0.03 and 12.2 ± 0.21 respectively. Responses to capsaicin were fast in onset and faded rapidly over a 30-min exposure period, whereas those to RTX were slow in onset and well maintained, an observation believed to reflect pharmacokinetic differences in the rate of penetration to the vanilloid receptor. Responses to both agonists showed mutual cross-desensitization and were antagonized by both the vanilloid-receptor antagonist capsazepine and the ion-channel blocker ruthenium red. The capsaicin analogue, olvanil failed to either mimic or antagonize capsaicin-evoked responses in the rat isolated vas deferens, an effect at variance with previous observations in other tissues.

The reason for these differences is unclear, but the possibility of multiple classes of receptor cannot at this stage be ruled out.

Capsaicin is the pungent agent found in capsicums and red chilli peppers. In man, local application of capsaicin produces irritation, a burning sensation coupled with a wheal-and-flare reaction. With repeated application, the intensity of the irritation gradually diminishes and the affected area becomes insensitive to a variety of noxious stimuli. This latter phase is known as desensitization and has stimulated an interest in capsaicin analogues as potential non-narcotic analgesics (for review see Craft & Porreca 1992; Dray 1992; Maggi 1992; Campbell et al 1993).

A specific receptor, termed the vanilloid receptor, has recently been identified on the cell membrane of primary afferent neurones which recognizes capsaicin and other natural pungent agents including the ultra-potent capsaicin analogue resiniferatoxin (RTX, Szallasi & Blumberg 1990). Studies have shown that stimulation of the vanilloid receptor results in the opening of a novel receptor-operated, non-specific cation channel, resulting in membrane depolarization and neuropeptide release (for review see Bevan & Docherty 1993).

Characterization of the vanilloid receptor has been severely hampered due to a lack of selective pharmacological tools. Available blockers of capsaicin-induced responses include the competitive antagonist capsazepine (Bevan et al 1991; 1992; Dickenson et al 1991; Dray et al 1991) and the inorganic dye ruthenium red (Amann & Maggi 1991). Initially, capsaicin itself was the only available agonist at the vanilloid receptor. More recently, however, both RTX (Szallasi & Blumberg 1990; 1992b) and olvanil (Sietsema et al 1988; Bettaney et al 1990; Dray et al 1990a) have been

shown to mediate their effects via this site. The present study uses these pharmacological tools to characterize the vanilloid receptor in the rat isolated vas deferens, a tissue previously shown to possess the vanilloid receptor (Maggi et al 1987; Santicioli et al 1988; Maggi et al 1993). The need to undertake such a complete characterization is illustrated by the recently reported differences in vanilloid receptor operational pharmacology and by the implications such data may have on the possible existence of vanilloid receptor subtypes (see Szallasi 1994). Preliminary findings have been published elsewhere (Wardle et al 1995).

Methods

Tissue preparation

Vas deferens were removed from male Wistar rats, 200–300 g, and placed in Krebs solution of the following composition (mM): NaCl 121·5, $CaCl_2$ 2·5, KH_2PO_4 1·2, KCl 4·7, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, glucose 5·6.

The whole length of the vas deferens was mounted under 0·5-g load in 10-mL tissue baths. Tissues were bathed in Krebs solution at 37°C gassed with 5% CO_2 in O_2. Vas deferens were stimulated electrically (0·2 Hz, 0·5 ms pulse width, supramaximal voltage) by means of two platinum wire electrodes suspended parallel to the tissue. Contractile responses were recorded isotonically and displayed on a Lectromed MT8P multitrace chart recorder. Tissues were allowed to equilibrate for 60 min (washing every 10 min), or until responses to electrical stimulation became consistent, before starting the experiment.

Construction of concentration–effect curves

Following equilibration, tissues were dosed cumulatively with increasing concentrations of capsaicin, RTX, olvanil

*Present address: Department of Pharmacology, University of Leeds, Leeds, UK.

Correspondence: K. A. Wardle, Department of Neurology Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, Essex, UK.

or calcitonin gene-related peptide (CGRP). The time course of agonist addition was determined by the rate of onset and time to plateau of the agonist response. With the exception of CGRP, a single agonist concentration–effect curve was constructed in each tissue. The effects of receptor antagonists or ion-channel blockers on the responses to capsaicin, RTX and olvanil were investigated in paired vas deferens from the same animal, following a 40-min equilibration period. The effects of antagonists on responses to CGRP were studied in the same tissue (the first curve in the absence, the second in the presence, of the appropriate concentration of antagonist).

In a separate series of experiments, the time course of agonist action was studied by adding a single concentration of the agonist under observation to the tissue and monitoring the time course of agonist effect over a 30-min observation period.

Desensitization studies were performed by incubating tissues with a single concentration of test drug for 40 min, washing out for 30 min, then constructing a concentration–effect curve to capsaicin in the normal way.

Analysis of results

Responses were expressed as a percent inhibition in the amplitude of electrically-evoked contractions. All agonist concentration–effect curves were fitted using a non-linear iterative fitting programme (Kaleidagraph, Synergy Software, PCS Inc, Reading, PA) according to the following relationship (Parker & Waud 1971):

$$E = M[A]^p/([A]^p + [K]^p) \qquad (1)$$

This relationship describes a curve with a maximum response M, and EC50 equal to K and a slope determined by the power p. [A] represents the agonist concentration and E is the response.

The ability of agonists to evoke inhibition in the amplitude of electrically-evoked contractions was expressed in terms of pEC50 values (relative to their own maxima) and in terms of a maximum response, relative to capsaicin (intrinsic activity, where intrinsic activity of capsaicin = 1·0). The potency relative to capsaicin was calculated from experiments
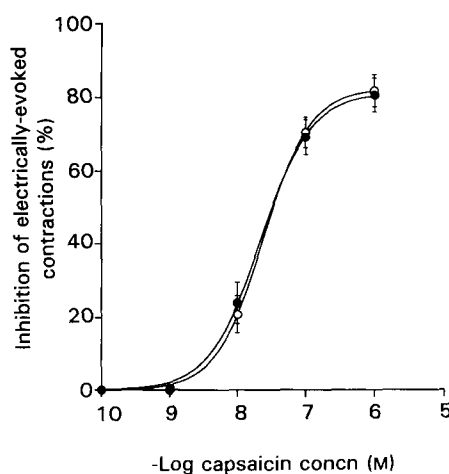
using paired vas deferens from the same rat (one vas used to construct a concentration–effect curve to capsaicin, the other used for the test agonist).

pA2 values for capsazepine versus capsaicin were determined according to the method of Arunlakshana & Schild (1959), with the effects of the antagonist being determined in paired tissues. Where antagonism was not competitive (as was the case with ruthenium red), the potency of the antagonist was estimated in terms of a pD_2' value, defined as the concentration of antagonist required to reduce by half the maximum response of the agonist.

All results are expressed in terms of mean ± s.e.m. for a number (n) of observations. Statistical analysis was performed by means of a Student's t-test for paired or unpaired data. A P value of less than 0·05 was taken to be significant.

Drugs used

The following drugs were used: capsaicin (Sigma), RTX (Sigma), olvanil (Procter and Gamble), capsazepine (Tocris Cookson), ruthenium red (RBI), rat CGRP (Sigma), tetrodotoxin (Sigma), prazosin (Sigma), α, β-methylene ATP (Sigma). Ruthenium red, tetrodotoxin, prazosin, α, β-methylene ATP and CGRP were dissolved in distilled water. Stock solutions (20 mM) of capsaicin, olvanil and capsazepine were dissolved in absolute ethanol and further diluted in water (capsaicin and capsazepine) or ethanol/water mixture (olvanil) to give the required concentration.

Results

General experiments

In the rat isolated vas deferens, electrical field stimulation (0·2 Hz, 0·5 ms pulse width, supramaximal voltage) evoked consistent twitch contractions which were abolished by TTX (1 μM, n = 4) and reduced by 63 ± 6% by prazosin (0·1 μM, n = 6). The remaining response was abolished by desensitization with α, β-methylene ATP (1 μM, n = 6) suggesting that the responses were predominately due to

Fig. 1. Concentration–effect curve to capsaicin in left (○) and right (●) vas deferens from a single rat. Each point represents the mean ± s.e.m. of 8 experiments.
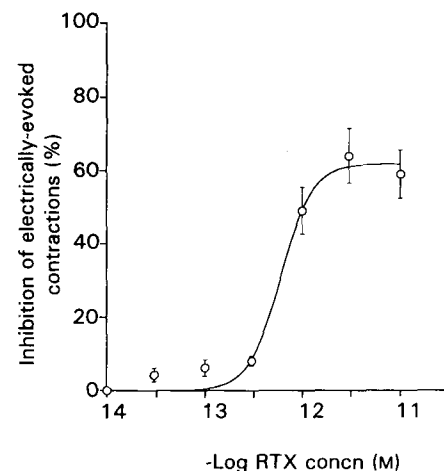

Fig. 2. Concentration–effect curve to RTX in the isolated vas deferens. Each point represents the mean ± s.e.m. of 8 experiments.

stimulation of noradrenergic and purinergic neurones (data not shown).

Agonist profile

Capsaicin (1–1000 nM, n = 16) evoked a concentration–dependent inhibition of electrically-evoked contractions with a pEC50 of 7·64 ± 0·01 and a maximum inhibition of 82 ± 1·0%. Within one animal, the responses to capsaicin were not significantly different in the two vas deferens (Fig. 1). Thus, the mean pEC50 for the right vas deferens was 7·62 ± 0·03 with a mean percent inhibition of electrically-evoked contractions of 82·3 ± 1·2% compared with 7·66 ± 0·03 and 81·1 ± 1·4%, respectively for the left vas deferens (n = 8 each). Following washout, attempts to evoke a second concentration–effect curve to capsaicin in the same tissue were unsuccessful, suggesting that the receptor becomes rapidly desensitized (results not shown).

RTX (0·1–100 pM, n = 8, Fig. 2) also inhibited the amplitude of electrically-evoked contractions in the rat isolated vas deferens. RTX was several orders of magnitude more potent than capsaicin in its effects (pEC50 12·2 ± 0·21) and was a partial agonist relative to capsaicin, producing a maximum inhibition of electrically-evoked contractions of 61·8 ± 3·1%. Responses to RTX were much more variable

-Log capsaicin concn (M)

Fig. 4. Concentration–effect curve to capsaicin following prior exposure to the vehicle for RTX (O, control) or 0·1 pM (●), 1 pM (□), 10 pM (■), 0·1 nM (▼), 1 nM (▲) and 10 nM (x) RTX. Each point represents the mean ± s.e.m. of 6 experiments.

than those to capsaicin. While the responses to capsaicin were rapid in onset (mean rate of onset at pEC50 concentration of 4·3 ± 0·1 min, Fig. 3A) and faded rapidly over a 30-min observation period, those to RTX were slow in onset (mean rate of onset at pEC50 concentration of 17·3 ± 0·2 min, Fig. 3B) and were well maintained over a 30-min observation period. Responses to capsaicin were reduced in a non-competitive manner following a 40-min incubation (followed by 30-min washout) with RTX (0·1 pM–1 nM, n = 6, Fig. 4). Similar results were observed when tissues were challenged with capsaicin (0·1–1000 nM, 40 min incubation followed by washout, n = 6) 30 min
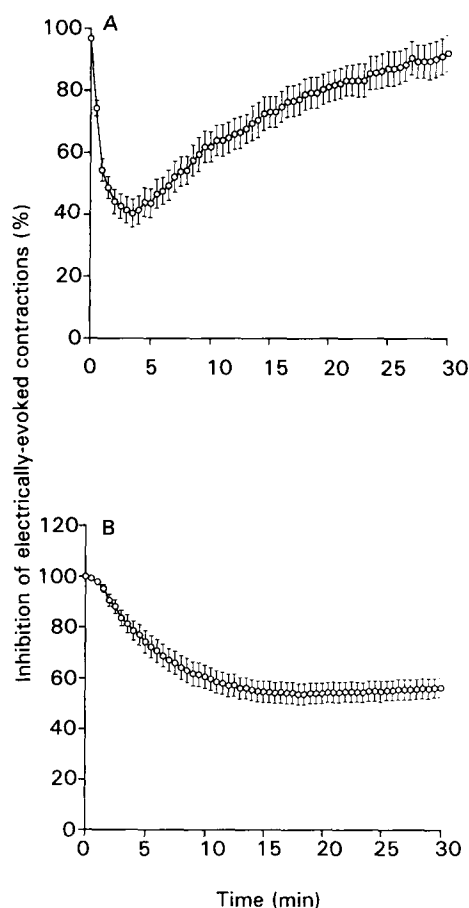

Time (min)

Fig. 3. Time course studies showing the effects of a single dose of capsaicin (30 nM, A) and RTX (1 pM, B) over a 30-min observation period. Each point represents the mean ± s.e.m. of 6 observations.
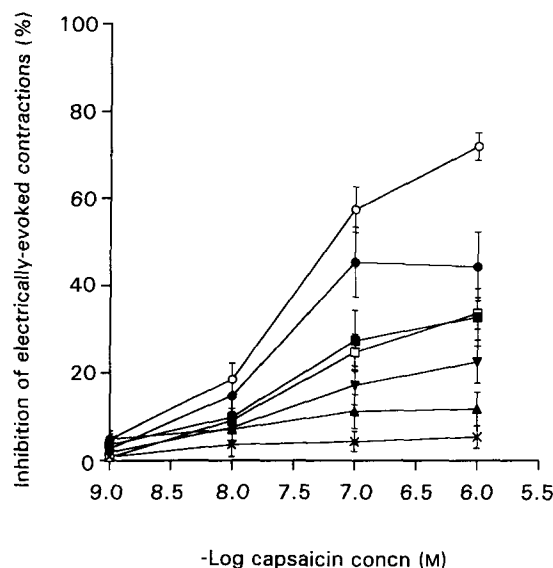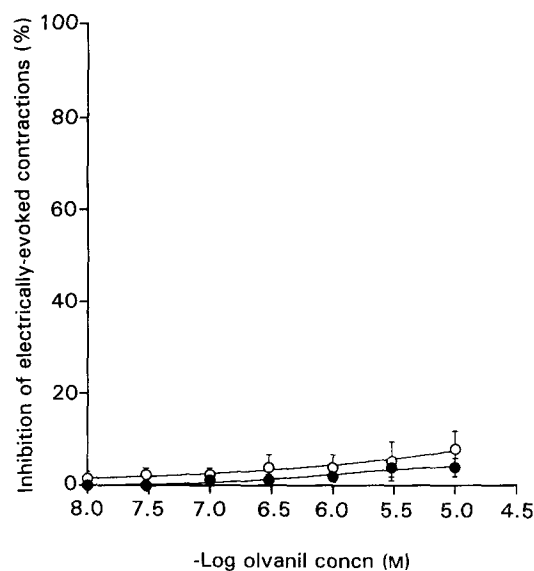

-Log olvanil concn (M)

Fig. 5. Concentration–effect curve to olvanil (O) and its solvent (●) in the rat isolated vas deferens. Each point represents the mean ± s.e.m. of 8 experiments.

before construction of the RTX concentration-effect curve (results not shown).

Olvanil (10 nM–10 μM, n = 8, Fig. 5) had no significant effect on the amplitude of electrically-evoked contractions. Following a 40-min incubation with 10 μM olvanil, the concentration-effect curve to capsaicin was not significantly modified (control pEC50 7·58 ± 0·05; 78·4 ± 3·6%

inhibition of electrically-evoked contractions, compared with a pEC50 of 7·46 ± 0·07 and 72·1 ± 6·3% inhibition in the presence of 10 μM olvanil, n = 6 each, data not shown).

Electrically-evoked contractions in the rat isolated vas deferens were inhibited in a concentration-dependent manner by CGRP (0·1–100 nM, n = 8). The mean pEC50 was calculated to be 8·33 ± 0·05, with a maximum inhibition of electrically-evoked contractions of 94 ± 3%. In contrast to the situation with capsaicin, it was possible to construct two consecutive concentration-effect curves to CGRP in the same tissue (Fig. 6A).

Antagonist studies

The concentration-effect curve to capsaicin was antagonized in a competitive manner by capsazepine (3–30 μM, n = 8, Fig. 7A), yielding an estimated pA_2 value of 5·7 ± 0·2. At these concentrations, capsazepine had no significant effect on the amplitude of electrically-evoked contractions. Ruthenium red (1–10 μM, n = 8) evoked a concentration-dependent inhibition in the amplitude of electrically-evoked contractions (2·1 ± 1·5% at 1 μM; 7·6 ± 2.3% at 3 μM and 35·0 ± 5·3% at 10 μM, data not shown) and
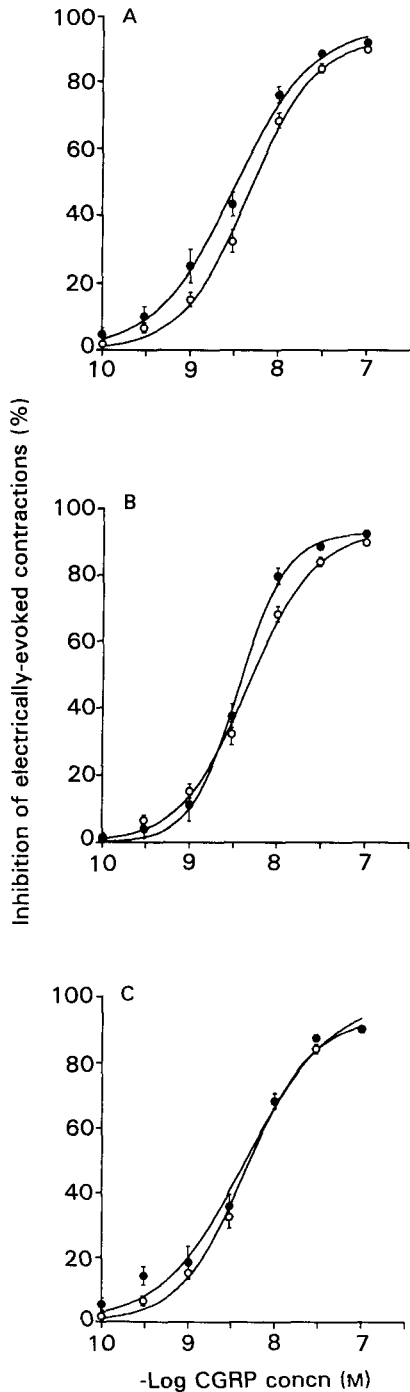

FIG. 6. Concentration-effect curves to CGRP in the rat isolated vas deferens. A: first (○) and second (●) concentration-effect curve to CGRP. B: in the absence (○) and presence (●) of 30 μM capsazepine. C: in the absence (○) and presence (●) of 10 μM ruthenium red. Each point represents the mean ± s.e.m. of 8 experiments.

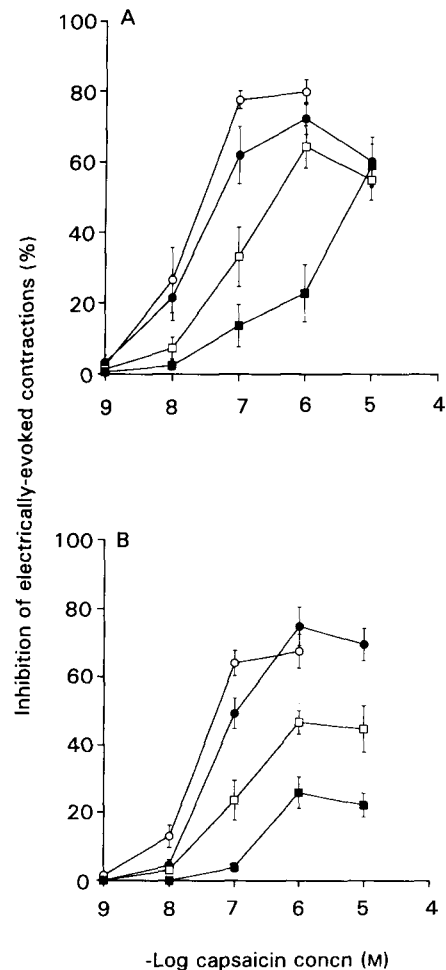FIG. 7. Concentration-effect curves to capsaicin in the absence (○) and presence of 3 (●), 10 (■) and 30 (□) μM capsazepine (Graph A) and 1 (●), 3 (■) and 10 (□) μM ruthenium red (Graph B). Each point represents the mean ± s.e.m. of 8 experiments.

subsequently antagonized capsaicin-evoked responses in an apparently non-surmountable manner (Fig. 7B).

Responses to RTX were also antagonized by capsazepine (10 μM) and ruthenium red (3 μM); however accurate analysis of this antagonism was not possible due to the slow equilibration rate of RTX which precluded examination of a full concentration–effect curve to this agonist. Examples of the antagonism evoked by capsazepine (10 μM, n = 6) and ruthenium red (3 μM, n = 6) are shown in Figs 8A and 8B, respectively.

Responses to CGRP (0·1–100 nM, n = 8) were not significantly affected by 30 μM capsazepine (Fig. 6B, n = 8) or 10 μM ruthenium red (Fig. 6C, n = 8).

Discussion

The Hungarian pharmacologist Nicholas Jancso was among the first researchers to propose the existence of a capsaicin-sensitive pain receptor expressed almost exclusively by primary afferent neurones (Jancso 1968). Confirmation that such a site existed, however, was to take almost another two decades and the identification of RTX as a high affinity capsaicin-like analogue (Szallasi & Blumberg 1990). Notwithstanding the elegant binding studies of Szallasi & Blumberg 1990, 1992a, a lack of potent, selective pharmacological tools has prevented full characterization of the capsaicin, or vanilloid receptor. In the present study some newly available

Fig. 8. Time course studies showing the response to a single dose of RTX (1 pM) over a 30-min observation period in the absence (○) and presence (●) of capsazepine (10 μM, A) and ruthenium red (3 μM, B). Each point represents the mean ± s.e.m. of 6 observations.

tools have been used to pharmacologically characterize the vanilloid receptor in a functional model, namely the rat isolated vas deferens.

Electrical stimulation of the intramural neurones in the rat isolated vas deferens evoked rapid twitch contractions which were blocked by a mixture of prazosin and α,β-methylene ATP, confirming earlier suggestions (Sneddon & Westfall 1984; McGrath 1987) that electrically-evoked contractions in this tissue were mediated by the co-release of noradrenaline and ATP.

In agreement with earlier studies (Maggi et al 1993), electrically-evoked contractions were inhibited in a concentration–dependent manner by the cumulative addition of capsaicin. With repeated application, the rat vas deferens became unresponsive to capsaicin, suggesting that desensitization of the vanilloid receptor had occurred. For this reason, only one concentration–effect curve to capsaicin (or other vanilloid-receptor agonists) was constructed in each tissue.

RTX also inhibited the amplitude of electrically-evoked contractions in the rat isolated vas deferens, although the responses differed from those to capsaicin in several ways. First RTX was several orders of magnitude more potent than capsaicin in its effects. Secondly, RTX was a partial agonist relative to capsaicin, producing a maximum inhibition of electrically-evoked contractions of $61\cdot8 \pm 3\cdot1\%$. Thirdly, responses to RTX were much more variable than those to capsaicin. Finally and most notably, while the responses to capsaicin were rapid in onset (mean rate of onset at pEC50 concentration of $4\cdot3 \pm 0\cdot1$ min) and faded rapidly over a 30-min observation period, those to RTX were slow in onset (mean rate of onset at pEC50 concentration of $17\cdot3 \pm 0\cdot2$ min) and were well maintained. Responses to capsaicin were reduced in a non-competitive manner following a pre-exposure to RTX, suggesting that both compounds mediated their effect via a common site of action.

Thus, in the present study, RTX was demonstrated to be around 30000 times more potent than capsaicin at exciting primary afferent neurones in the rat isolated vas deferens. This observation is comparable with that observed in a previous study in the same preparation (e.g. Maggi et al 1990). The potency of RTX relative to capsaicin, however, has been reported to vary from one model to another. For example, RTX has previously been shown to be 1000–10000 times more potent than capsaicin in exciting capsaicin-sensitive afferent nerves in the rat isolated vas deferens, but was only 3 times more potent at exciting primary afferent neurones in the rabbit ear (Maggi et al 1990). Similarly, Szallasi & Blumberg (1989) have previously noted that RTX was only two times more potent than capsaicin in the rat eye-wiping assay but was several orders of magnitude more potent than capsaicin in inducing neurogenic inflammation and hypothermia. The explanation for the marked differences in potency ratios between capsaicin and RTX is unclear. Some workers favour the hypothesis that multiple classes of receptor exist with differential selectivity between RTX and capsaicin (Blumberg et al 1993). However, results from the present as well as previous (Maggi et al 1990) studies indicate that the penetration rate of RTX in tissues may be slower than that of capsaicin. This is clearly evident when comparing the time course of twitch inhibition
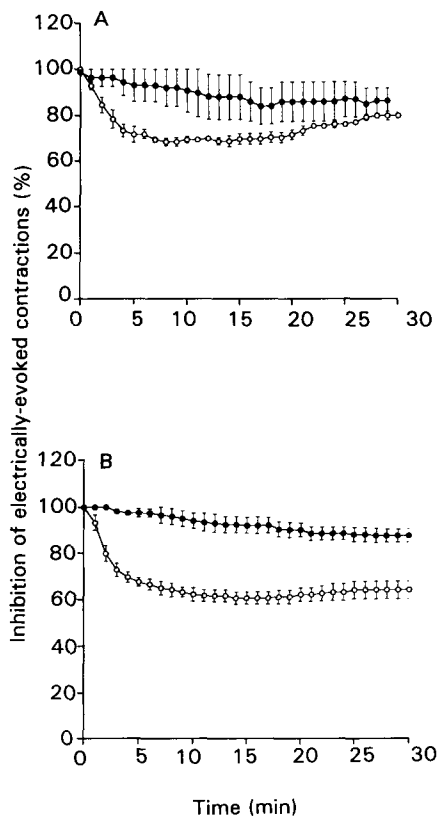
produced by the two compounds in the rat isolated vas deferens. If these differences in onset of effect are related to the physicochemical factors which limit the rate of penetration of RTX to the vanilloid receptor, then it becomes clear that estimates of the potency of RTX may be misinterpreted due to poor experimental design, in which equilibrium conditions have not been achieved. Thus while claims of multiple vanilloid receptors cannot be excluded, pharmacokinetic differences must first be ruled out.

The capsaicin analogue olvanil has previously been reported to display a similar antinociceptive potency to capsaicin in-vivo (Sietsema et al 1988; Campbell et al 1989) and to be approximately one-sixth as potent as capsaicin at evoking CGRP release from rat dorsal spinal cord (Banner & Bowen 1994), suggesting that it is an agonist at the central vanilloid receptor. In the present study, however, olvanil failed to either mimic or antagonize the responses to capsaicin at the peripheral vanilloid receptors in the rat isolated vas deferens. A similar lack of activity has previously been reported at peripheral nociceptors in the neonatal rat spinal cord-tail preparation (Dray et al 1990a). The reason for these discrepancies is at present unclear, but may reflect a peripheral-central difference in the vanilloid receptor. Further work must, however, be carried out before such a suggestion can be ratified or rejected.

The inhibition of electrically-evoked contractions seen with capsaicin and other vanilloid receptor agonists was mimicked by the peptide CGRP (see Santicioli et al 1988; Maggi et al 1993). This inhibitory action of CGRP on the electrically-evoked contractions has previously been reported to be mainly due to a presynaptic inhibitory mechanism on noradrenergic nerve terminals in the vas deferens (Ohhashi & Jacobowitz 1985). Since immunofluorescence studies in the rat periphery have demonstrated a wide distribution of CGRP-like immunoreactivity in visceral organs (Rosenfeld et al 1983), it has been concluded that the inhibition of twitch amplitude seen with capsaicin is ascribable to an action on sensory nerves and the subsequent release of CGRP (Maggi et al 1987). While it was possible to construct only a single concentration–effect curve to capsaicin in each vas deferens, multiple curves could be constructed to CGRP, suggesting that the desensitization seen with capsaicin was not at the level of the CGRP receptor.

Capsazepine produced a rightward displacement of the concentration–effect curve to capsaicin with no significant depression of the maximum response and no effect of baseline activity, confirming that this compound is a competitive antagonist at the vanilloid receptor (Bevan et al 1991, 1992; Dickenson et al 1991; Dray et al 1991; Maggi et al 1993). Ruthenium red, on the other hand, in addition to displacing the concentration–effect curve to the right, produced a concomitant reduction in the maximum response, an effect suggestive of a non-competitive mechanism of action. At the concentration required to antagonize capsaicin-evoked responses, ruthenium red also inhibited the amplitude of electrically-evoked contractions, confirming earlier observations that ruthenium red is not a selective compound (see Amann & Maggi 1991 for review). The exact mechanism by which ruthenium red blocks capsaicin-evoked responses is unclear, but is believed to act at the membrane level to interfere with the opening of the capsaicin-operated cation

channel (Dray et al 1990b). Thus, Dray et al (1990b) have reported that the blockade of capsaicin-activated single-channel activity by ruthenium red had different characteristics from those expected from a typical ion-channel blocker. Instead it has been suggested that ruthenium red may interfere with the binding site of capsaicin or with the receptor-channel coupling mechanism (Dray et al 1990b). The results seen in the present study would be consistent with either of these effects.

The failure of capsazepine or ruthenium red to significantly affect the responses to CGRP in the rat vas deferens would appear to rule out a post-junctional effect of either of these compounds at the level of the CGRP receptor. Responses to RTX, on the other hand, were antagonized by both capsazepine and ruthenium red, adding support to the idea that capsaicin and RTX act via a common receptor (Szallasi & Blumberg 1990).

In conclusion, the present study has aimed to further characterize the vanilloid receptor in the rat isolated vas deferens using the available pharmacological tools. The results have demonstrated a clear difference in the rate of onset of responses between capsaicin and RTX. This effect is likely to be due to pharmacokinetic differences, but the existence of multiple classes of receptor cannot be excluded. Thus, the lack of intrinsic activity of olvanil in the rat vas deferens is of particular interest in view of previous reports (Sietsema et al 1988; Campbell et al 1989; Banner & Bowen 1994) that this compound is a full agonist in other systems. Elucidation of this point awaits further investigation.

References

Amann, R., Maggi, C. A. (1991) Ruthenium red as a capsaicin antagonist. Life Sci. 49: 849–856

Arunlakshana, O., Schild, H. O. (1959) Some quantitative uses of drug antagonism. Br. J. Pharmacol. Chemother. 14: 48–58

Banner, S. E., Bowen, W. P. (1994) The effects of vanilloids in a novel in vitro filtration assay for neurotransmitter release in rat dorsal spinal cord. Br. J. Pharmacol. 113: 38P

Bettaney, J., Dickenson, A., Dray, A., Hughes, C. (1990) Antinociception induced by the capsaicin analogue, olvanil: peripheral and central sites of action studied in vivo in adult rats and in vitro using neonatal rats. J. Physiol. 424: 60P

Bevan, S. J., Docherty, R. J. (1993) Cellular mechanisms of the action of capsaicin In: Wood, J. (ed.) Capsaicin in the Study of Pain. Academic Press, London, pp 27–44

Bevan, S. J., James, I. F., Rang, H. P., Shah, K., Yeats, J. C. (1991) The development of a capsaicin antagonist for the sensory neurone excitant, capsaicin. Br. J. Pharmacol. 102: 77P

Bevan, S. J., Hothi, S., Hughes, G., James, I. F., Rang, H. P., Shah, K., Walpole, C. S. J., Yates, J. C. (1992) Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. Br. J. Pharmacol. 107: 544–552

Blumberg, P. M., Szallasi, A., Acs, G. (1993) Resiniferatoxin—an ultrapotent capsaicin analogue. In: Wood, J. (ed.) Capsaicin in the Study of Pain. Academic Press, London, pp 45–62

Campbell, E. A., Dray, A., Perkins, M. N. (1989) Comparison of capsaicin and olvanil as antinociceptive agents in vivo and in vitro. Br. J. Pharmacol. 98: 907P

Campbell, E. A., Bevan S., Dray, A. (1993) Clinical applications of capsaicin and its analogues. In: Wood, J. (ed.) Capsaicin in the Study of Pain. Academic Press, London, pp 255–272

Craft, R. A., Porreca, F. (1992) Therapeutic potential of capsaicin-like molecules. Treatment parameters of desensitisation to capsaicin. Life Sci. 51: 1767–1775

Dickenson, A. H., Dray, A., Hughes, G. A., Walpole, C. S. J. (1991) The selective antagonist capsazepine inhibits capsaicin induced antinociception: electrophysiological studies in rodents. Br. J. Pharmacol. 102: 79P

Dray, A. (1992) Therapeutic potential of capsaicin-like molecules. Mechanism of action of capsaicin-like molecules on sensory neurones. Life Sci. 51: 1759–1765

Dray, A., Bettaney, J., Rueff, A., Walpole, C., Wrigglesworth, R. (1990a) NE-19550 and NE-21610, antinociceptive capsaicin analogues: studies on nociceptive fibres of the neonatal rat tail in vitro. Eur. J. Pharmacol. 181: 289–293

Dray, A., Forbes, C. A., Burgess, G. M. (1990b) Ruthenium red blocks the capsaicin-induced increase in intracellular calcium and activation of membrane currents in sensory neurones as well as the activation of peripheral nociceptors in vitro. Neurosci. Lett. 100: 52–59

Dray, A., Campbell, E. A., Hughes, G. A., Patel, I. A., Perkins, M. N., Rang, H. P., Rueff, A., Seno, N., Urban, L., Walpole, C. S. J. (1991) Antagonism of capsaicin-induced activation of C fibres by a selective capsaicin antagonist capsazepine. Br. J. Pharmacol. 102: 78P

Jancso, N. (1968) Desensitisation with capsaicin and related acylamides as tools for studying the function of pain receptors. In: Lin, K., Armstrong, D., Pardo, E. G. (eds) Pharmacology of Pain. Pergamon Press, Oxford, vol 9, pp 33–55

Maggi, C. A. (1992) Therapeutic potential of capsaicin-like molecules: studies in animals and humans. Life Sci. 51: 1777–1781

Maggi, C. A., Giuliani, S., Santicioli, P., Meli, A. (1987) Capsaicin-induced inhibition of motility of the rat isolated vas deferens: do multiple neuropeptides mediate the visceromotor effects of capsaicin? J. Auton. Pharmacol. 7: 243–255

Maggi, C. A., Patacchini, R., Tramontana, M., Amann, R., Guiliani, S., Santicioli, P. (1990) Similarities and differences in the action of resiniferatoxin and capsaicin on central and peripheral endings of primary sensory neurones. Neurosci. 37: 531–539

Maggi, C. A., Bevan, S., Walpole, C. S. J., Rang, H. P., Giuliani, S. (1993) A comparison of capsazepine and ruthenium red as capsaicin antagonists in the rat isolated urinary bladder and vas deferens. Br. J. Pharmacol. 108: 801–805

McGrath, J. C. (1987) Adrenergic and 'non-adrenergic' components of the contractile response of the rat vas deferens to a single indirect stimulus. J. Physiol. 283: 23–39

Ohhashi, T., Jacobowitz, D. M. (1985) Effects of calcitonin gene-related peptide on the neuroeffector mechanism of sympathetic nerve terminals in rat vas deferens. Peptides 4: 987–991

Parker, R. B., Waud, D. R. (1971) Pharmacological estimation of drug receptor dissociation constants. Statistical evaluation. I. Agonists. J. Pharmacol. Exp. Ther. 177: 1–12

Rosenfeld, M. G., Mermod, J. J., Amara, S. G., Swanson, L. W., Sawchenko, P. E., River, J., Vale, W. W., Evans, R. M. (1983) Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 304: 129–135

Santicioli, P., Maggi, C. A., Geppetti, P., Del Bianco, E., Theodorosson, E., Meli, A. (1988) Release of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) from organs of the genitourinary tract in rats. Neurosci. Lett. 92: 197–201

Sietsema, W. K., Berman, E. F., Farmer, R. W., Maddin, C. S. (1988) The antinociceptive effect and pharmacokinetics of olvanil following oral and subcutaneous dosing in the mouse. Life Sci. 43: 1385–1391

Sneddon, P., Westfall, D. P. (1984) Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea-pig vas deferens. J. Physiol. 347: 561–580

Szallasi, A. (1994) The vanilloid (capsaicin) receptor: receptor types and species differences. Gen. Pharmacol. 25: 223–243

Szallasi, A., Blumberg, P. M. (1989) Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analogue of capsaicin, the irritant constituent in red pepper. Neurosci. 30: 515–520

Szallasi, A., Blumberg, P. M. (1990) Specific binding of resiniferatoxin, an ultrapotent capsaicin analogue, by dorsal root ganglion membranes. Brain Res. 524: 106–111

Szallasi, A., Blumberg, P. M. (1992a) Vanilloid receptor loss in rat sensory ganglia associated with long term desensitisation to resiniferatoxin. Neurosci. Lett. 136: 51–54

Szallasi, A., Blumberg, P. M. (1992b) Resiniferatoxin. In: Conn, P. M. (ed.) Methods in Neuroscience. Academic Press, Orlando, Florida, vol 8, pp 368–380

Wardle, K. A., Furey, G., Sanger, G. J. (1995) Further characterisation of the vanilloid receptor in the rat isolated vas deferens. Br. J. Pharmacol. 115: 144P