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Binding of neuroleptic drugs (trifluoperazine and rimcazole) to vanilloid receptors in porcine dorsal horn

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Abstract

Neuroleptic drugs were reported to modulate [^{3}H]resiniferatoxin binding to vanilloid receptors in the spinal cord, with marked differences between rat and man. In the present study, we have used a [^{3}H]resiniferatoxin binding assay using porcine dorsal horn membranes to explore further species differences in the interaction of neuroleptic drugs at spinal vanilloid receptors. Specific binding of 13 pM [^{3}H]resiniferatoxin to porcine dorsal horn membranes (corresponding to a 7% fractional receptor occupancy) was affected by trifluoperazine in a bi-phasic fashion, with an initial 90% enhancement of binding preceding inhibition: a fit to the modified Hill equation yielded a cooperativity index of 1.8 and a K_i of 5 μM. Under similar conditions, rimcazole, by contrast, had a monophasic effect: it enhanced but, up to 100 μM, did not inhibit [^{3}H]resiniferatoxin binding. These results are in accord with previous findings in human spinal cord but contrast with those in the rat. In experiments in which the concentration of [^{3}H]resiniferatoxin was varied, 20 μM trifluoperazine reduced the B_{max} by 33% (from 181 $\pm$ 9 fmol/mg protein to 121 $\pm$ 5 fmol/mg protein) without a measurable change in affinity or cooperativity. In parallel experiments, by contrast, neither capsaicin nor capsazepine (both at a concentration of 10 μM) affected the B_{max} or cooperativity but, as expected, reduced the affinity from 61 $\pm$ 8 pM to 120 $\pm$ 11 pM or to 101 $\pm$ 7 pM, respectively. Whereas vanilloid receptor agonists (resiniferatoxin and capsaicin) affected [^{3}H]resiniferatoxin binding at low (approximately 7%) fractional receptor occupancies by the radioligand in a bi-phasic fashion, the competitive vanilloid receptor antagonist capsazepine failed to induce the initial binding enhancement. Thus, capsazepine appears to bind to vanilloid receptors in a non-cooperative fashion, or at least with much reduced positive cooperativity in this system. The mechanism by which neuroleptic drugs modulate resiniferatoxin binding is yet to be clarified and is clearly complicated as well as species-dependent; nonetheless, the reduced B_{max} at higher concentrations suggests that it may at least in part be non-competitive.

Keywords: [^{3}H]Resiniferatoxin; Vanilloid receptor; Capsazepine; Neuroleptic; Cooperative binding

1. Introduction

Neuroleptic drugs are in use on an empirical basis to alleviate neuropathic pain syndromes (Portenoy, 1992). It is not implausible that the analgesic action of these drugs is partly due to their antidepressant effects but a direct action on pain pathways is also possible (Hollister, 1992). Opioid receptors may be obvious targets for such an action. Neuropathic pain, however, usually responds less well to opioid than to neuroleptic drugs (Arner and Myerson, 1988; Portenoy et al., 1990), thus the involvement of opioid receptors in the analgesic action of neuroleptic agents is not very likely.

Capsaicin is one of the very few agents capable of relieving neuropathic pain (see Lynn, 1990; Szolcsanyi, 1991 for reviews). A receptor for capsaicin and related compounds was recently demonstrated by the specific binding of [^{3}H]resiniferatoxin (Szallasi and Blumberg, 1990a), an ultrapotent capsaicin analog (Szallasi and Blumberg, 1989), and then further substantiated by the development of a competitive capsaicin antagonist, called capsazepine (Bevan et al., 1992). This recognition site, referred to as the vanilloid receptor (Szallasi and Blumberg, 1990b; Szallasi, 1994), represents an attractive possible target to explore for compounds which alleviate neuro-

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pathic pain by an essentially unknown mechanism. In fact, several years ago the phenothiazine antipsychotic drug trifluoperazine was found to inhibit capsaicin-induced Ca^{2+} uptake by cultured sensory neurons (Wood et al., 1988). This action was, however, at that time attributed to the well-known calmodulin antagonist effect of trifluoperazine (Roufogalis et al., 1983) and the possibility of a direct interaction at vanilloid receptors was not evaluated.

Most recently, trifluoperazine and related neuroleptic drugs have been found to modulate [^{3}H]resiniferatoxin binding by human and rat vanilloid receptors in the spinal cord (Acs et al., 1995). Also, similar concentrations of trifluoperazine were shown to influence capsaicin-induced Ca^{2+} uptake by cultured rat dorsal root ganglion neurons (ibidem). Based on these observations, a possible hypothesis is that trifluoperazine may enhance the binding of a putative endogenous vanilloid to its receptor and thereby may accelerate receptor desensitization. Since desensitization to vanilloids is known to suppress the perception of neuropathic pain (see Szolcsanyi, 1991; Szallasi and Blumberg, 1993 for reviews), this hypothesis might provide a mechanism by which neuroleptics and tricyclic antidepressants are beneficial in such syndromes.

Vanilloid receptors show marked species-related differences in binding properties (Blumberg et al., 1993; Szallasi and Blumberg, 1993; Szallasi, 1994). Therefore, it is hardly unexpected that neuroleptic drugs interfere with resiniferatoxin binding to vanilloid receptors in 'fresh' rat and post-mortem human spinal cord specimens in very different manners (Acs et al., 1995). For example, rimcazole inhibits but does not enhance resiniferatoxin binding to vanilloid receptors in the rat, whereas in human spinal cord the situation is the reverse: rimcazole-induced binding enhancement is not followed by inhibition (ibidem). This study may imply that the rat is not a suitable model to study the interaction of neuroleptics at spinal vanilloid receptors as a possible mechanism for their adjuvant analgesic action, however, it does not rule out the possibility that the observed differences are not really species-related but are due to post-mortem changes in the human spinal cord samples.

The pig is frequently used to study the biological spectrum of actions of capsaicin (Franco-Cereceda and Lundberg, 1989; Matran et al., 1990; Alving et al., 1991; Stjärne et al., 1994). Also, vanilloid receptors in the pig spinal cord can be readily examined by membrane binding (Szallasi and Blumberg, 1991; Acs and Blumberg, 1994) or visualized by [^{3}H]resiniferatoxin autoradiography (Szallasi et al., 1994). We have, therefore, decided to characterize the interaction of trifluoperazine and rimcazole at specific [^{3}H]resiniferatoxin binding sites in the dorsal horn of porcine spinal cord and explore further possible species-related differences in the binding of neuroleptic drugs to vanilloid receptors in the spinal cord. For a better understanding of the binding mechanism(s) of neuroleptics to vanilloid receptors, inhibition by capsaicin and capsazepine of [^{3}H]resiniferatoxin binding was examined in parallel experiments.

2. Materials and methods

2.1. Tissues

Young domestic pigs, weighing 25–30 kg, were killed by an overdose of pentobarbital given i.v. A mid-thoracic segment of the spinal cord was removed and quickly frozen on dry ice. Spinal cord samples removed from Sprague-Dawley rats (females, 200–250 g body weight) killed under CO_2 anesthesia were also used. In some experiments, human spinal cord specimens removed approximately 48 h after clinical death (kindly donated by the Department of Forensic Medicine, Karolinska Institute, Stockholm, Sweden) were employed.

The use of human and animal tissues was approved by the appropriate institutional ethical committees.

2.2. Preparation of membranes for binding experiments

A partially purified membrane preparation was obtained as described previously (Szallasi and Blumberg, 1991). Briefly, dorsal horns were dissected out from frozen pig spinal cord discs and then disrupted with the aid of a Polytron tissue homogenizer in ice-cold buffer A (pH 7.4), which contained (in mM) $CaCl_2$, 0.75; $MgCl_2$, 2; NaCl, 5.8; KCl, 5; sucrose, 137; and Hepes, 10. Tissue homogenates were first centrifuged for 10 min at $1000 \times g$ (4°C), the pellets were discarded and the supernatants were further centrifuged for 30 min at $35\,000 \times g$. Pellets from the second centrifugation were resuspended in buffer A at an approximate protein concentration of 1 mg/ml, and then stored at −70°C until assayed. The protein content of the preparations was determined using a Bio-Rad kit (Bio-Rad Laboratories, Richmond, CA, USA). Membranes from dissected human dorsal horns or from whole rat spinal cords were obtained by a similar procedure.

2.3. Membrane binding experiments using [^{3}H]resiniferatoxin

Binding assays were carried out as described (Szallasi et al., 1992). The assay mixtures (a final volume of 500 μl) contained 70–80 μg aliquots of the membrane protein, buffer A, [^{3}H]resiniferatoxin, non-radioactive ligands, and 0.25 mg/ml bovine serum albumin (Cohn fraction V; Sigma, St. Louis, MO, USA) included to stabilize ligands in the aqueous solution. Non-specific binding was defined as that occurring in the presence of 100 nM non-radioactive resiniferatoxin. Binding was either analyzed in the presence of increasing concentrations of [^{3}H]resiniferatoxin in the approximate range of 12–400 pM, or in the presence of fixed concentrations of the radioligand (13 pM

and 60 pM, which correspond to approximately 7% and 50% fractional receptor occupancies, respectively), and various concentrations of competing ligands. The assay mixtures were set up on ice; the binding reaction was initiated by transferring the tubes into a 37°C water bath; and then terminated by chilling the assay mixtures on ice following a 60 min incubation at 37°C. Non-specific binding was reduced by adding 100 μg of α_1-acid glycoprotein, a plasma protein which binds resiniferatoxin even at ice temperature (Szallasi et al., 1992), to each tube. Bound and free [^{3}H]resiniferatoxin were separated by pelleting the membranes in a Beckman 12 microfuge (Beckman Instruments AB, Stockholm, Sweden), and then quantitated by scintillation counting. Under these conditions, specific binding represented at least 70% (at 400 pM [^{3}H]resiniferatoxin) of the total; at 13 pM [^{3}H]resiniferatoxin, specific binding exceeded 90% of the total.

2.4. Analysis of binding data

Specific bound values were calculated using measured total and non-specific binding and the partition coefficient of [^{3}H]resiniferatoxin between the aqueous phase and the membranes. Data from saturation binding experiments were fitted to the allosteric Hill equation (Endrenyi et al., 1975) using the computer program FitP (Biosoft, Cambridge, UK). Data from experiments in which binding of 13 pM or 50 pM of [^{3}H]resiniferatoxin was examined in the presence of various concentrations of non-radioactive ligands were analyzed by a computer fit to the modified Hill equation (Monod et al., 1965; Davis et al., 1977) as described previously (Acs and Blumberg, 1994).

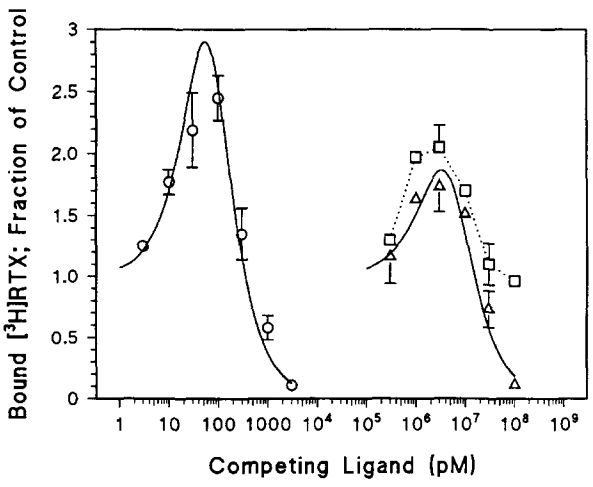

Fig. 1. Enhancement/inhibition of specific binding of 13 pM [^{3}H]resiniferatoxin to porcine dorsal horn of the spinal cord membranes by non-radioactive resiniferatoxin (circles), trifluoperazine (triangles) or rimcazole (squares). At this concentration, [^{3}H]resiniferatoxin occupies approximately 7% of the specific binding sites in the absence of other ligands. Note that both non-radioactive resiniferatoxin and trifluoperazine affect [^{3}H]resiniferatoxin binding in a bi-phasic fashion, with an initial binding enhancement preceding inhibition; rimcazole, by contrast, has a monophasic effect: it enhances but does not inhibit binding. Moreover, observe that enhancement of [^{3}H]resiniferatoxin binding by either neuroleptic drug is less than that produced by non-radioactive resiniferatoxin. Theoretical curves (solid lines) were generated using the modified Hill equation (see Methods). Points are mean values of triplicate assays from a single experiment; error bars represent S.E.M. Two additional experiments gave similar results. Binding parameters are summarized in Table 1. As shown, affinity for resiniferatoxin is 65 pM; cooperativity index for resiniferatoxin is 2.2; affinity for trifluoperazine is 5 μM; cooperativity index for trifluoperazine is 1.8.

2.5. [^{3}H]Resiniferatoxin autoradiography

Specific [^{3}H]resiniferatoxin binding sites in the pig spinal cord were visualized according to a published protocol (Szallasi et al., 1994). Briefly, 14 μM cryostat sections mounted onto slides precoated with chrome alum/gelatin were incubated at 37°C with 1 nM [^{3}H]resiniferatoxin in buffer A supplemented with 1 mg/ml bovine serum albumin in the absence or presence of non-radioactive ligands: 1 μM non-radioactive resiniferatoxin (background binding), 10 μM capsaicin, 10 μM capsazepine, 100 μM trifluoperazine, or 100 μM rimcazole. The binding reaction was terminated by rinsing the slides with ice-cold 20 mM Tris-Cl buffer (pH 7.4) following a 60 min incubation at 37°C. Slides were then washed 3 times with the above buffer supplemented with 0.1% (w/v) bovine serum albumin. After a dip into ice-cold distilled water, slides were dried in a stream of cold air and then exposed to Amersham Hyperfilm-^{3}H for 6 weeks. Quantitative analysis of relative gray levels was performed in the superficial dorsal horn (laminae I-II) of both sides using 8 sections for each experimental group. Images captured by a Nikon lens connected to a Quick Capture frame grabber board (Data Translation, Marlboro, MA, USA) were analyzed using a Macintosh PC with the Image Software 1.52 written by Dr. Wayne Raspband (NIHM, Bethesda, MD, USA). The mean grey level determined in the non-specific autoradiograms (background) was subtracted from the grey levels measured in the absence or presence of capsaicin, capsazepine, trifluoperazine or rimcazole, respectively (specific labelling); gray levels determined in the presence of the above compounds were expressed as percentages of grey levels measured under control conditions which were set to 100%. Data were assessed using the two-tailed unpaired *t*-test.

2.6. Materials

[^{3}H]Resiniferatoxin (specific activity = 37 Ci/mmol) was synthesized by the Chemical Synthesis and Analysis Laboratory, NCI-FCRDC, Frederick, MD, USA. Non-radioactive resiniferatoxin was from LC Laboratories (Woburn, MA, USA). Capsazepine was a gift of the Sandoz Institute for Medical Research, London, UK. Rimcazole was purchased from Research Biochemicals International (Natick, MA, USA). All the other chemicals, including trifluoperazine and capsaicin, were from Sigma

Chemical Co. (St. Louis, MO, USA) and were of the highest quality available.

3. Results

As predicted by the modified Hill equation for positive cooperative binding (Davis et al., 1977), non-radioactive resiniferatoxin affected the binding of 13 pM [^{3}H]resiniferatoxin (corresponding to 7% receptor occupancy in the absence of other ligands) to porcine dorsal horn membranes in a bi-phasic fashion, with an initial 140% increase in binding (measured value) preceding inhibition (Fig. 1). A fit of the measured specific bound values to the above equation yielded a cooperativity index of 2.2 ± 0.1 and an affinity of 65 ± 3 pM (mean $\pm$ S.E.M.; 3 determinations). In the presence of 60 pM [^{3}H]resiniferatoxin, the approximate K_d value from saturation binding experiments (see below), inhibition by non-radioactive resiniferatoxin followed a monophasic, sigmoidal inhibition curve (not shown).

In parallel experiments, trifluoperazine likewise affected resiniferatoxin binding in a bi-phasic fashion in the presence of 13 pM [^{3}H]resiniferatoxin (Fig. 1), and in a monophasic fashion in the presence of 60 pM [^{3}H]resiniferatoxin (not shown). For trifluoperazine, a computer fit to the modified Hill equation gave a Hill coefficient of 1.8 ± 0.2 and a K_i of 5 ± 4 μM (mean $\pm$ S.E.M.; 3 determinations). Rimcazole, by contrast, up to a concentration of 100 μM did not inhibit resiniferatoxin binding to porcine dorsal horn membranes, although it enhanced binding of 13 pM [^{3}H]resiniferatoxin to a similar level as did trifluoperazine (Fig. 1). This difference between trifluoperazine and rimcazole was confirmed by [^{3}H]resiniferatoxin autoradiography (Fig. 2) thus presumably it is not an artifact produced by tissue homogenization. Quantitatively, a significant (two-tailed unpaired t-test, $P < 0.001$), 22% decrease was observed in the density of autoradiographic labelling of porcine spinal cord sections by [^{3}H]resiniferatoxin in the presence of 100 μM trifluoperazine, whereas a similar concentration of rimcazole had no measurable effect on autoradiographic labelling. In accord with

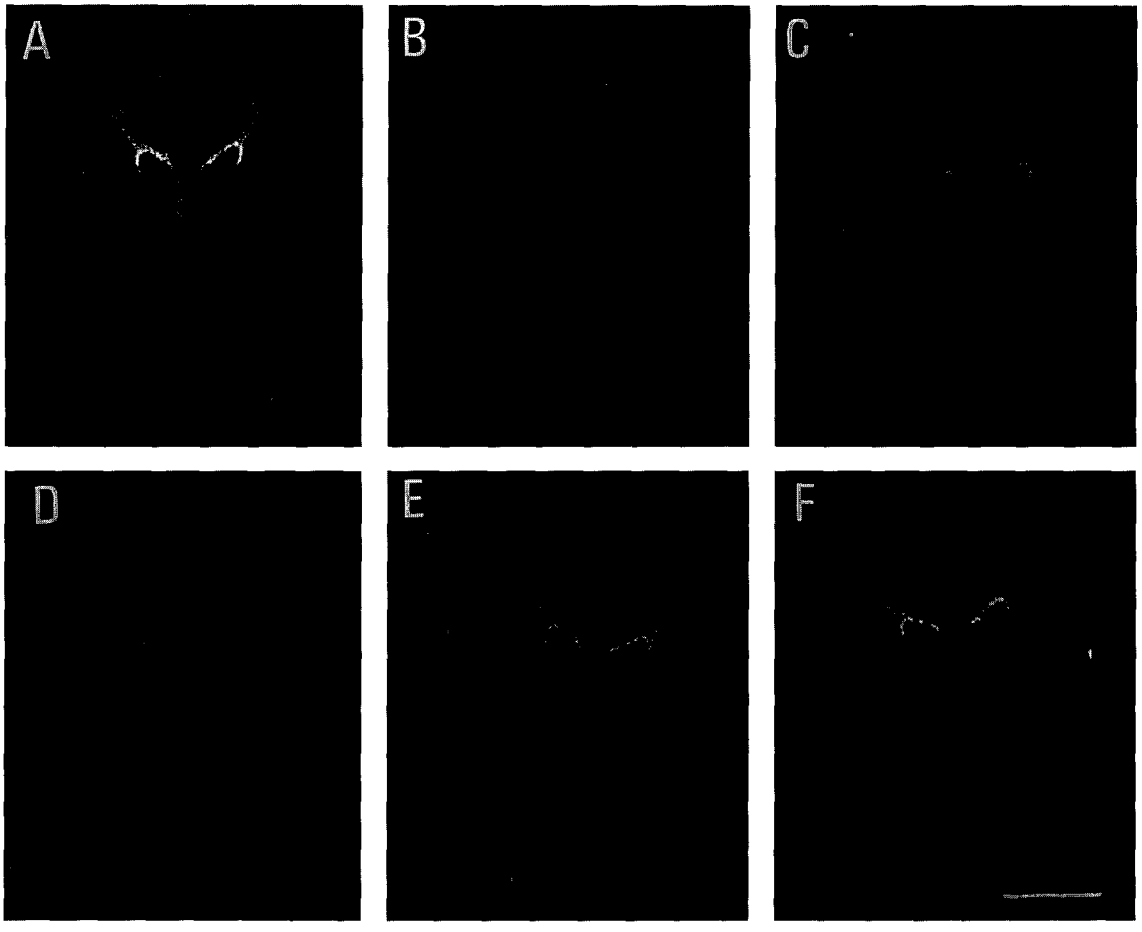

Fig. 2. Film autoradiograms of cryostat sections of pig spinal cord that were incubated with 1 nM [^{3}H]resiniferatoxin in the absence (panel A) or presence of 1 μM non-radioactive resiniferatoxin (panel B; background), 10 μM capsaicin (panel C), 10 μM capsazepine (panel D), 100 μM trifluoperazine (panel E) or 100 μM rimcazole (panel F). Observe that, unlike trifluoperazine, rimcazole up to 100 μM fails to inhibit the [^{3}H]resiniferatoxin labelling of porcine spinal cord sections. Marker indicates 14 mm.

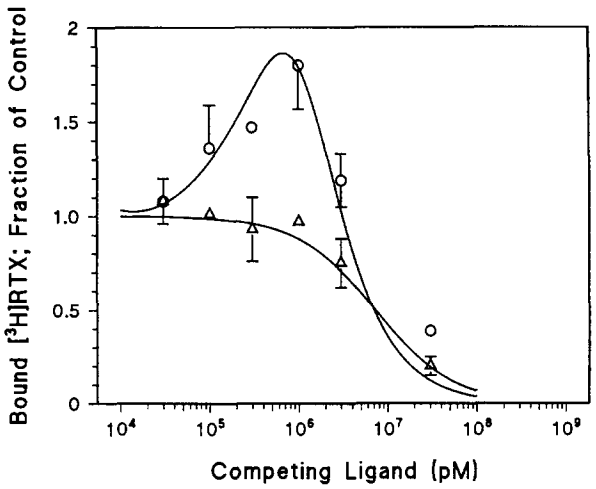

Fig. 3. Enhancement/inhibition of specific binding of 13 pM [^{3}H]resiniferatoxin (corresponding to 7% fractional receptor occupancy) to porcine dorsal horn of the spinal cord membranes by capsaicin (circles) or capsazepine (triangles). Observe that capsaicin has a bi-phasic effect on resiniferatoxin binding whereas inbition by capsazepine lacks the initial binding enhancement phase. Curves are theoretical, and were created using the modified Hill equation (see Methods). Points are mean values of triplicate assays from a single experiment; error bars represent S.E.M. Two additional experiments gave similar results.

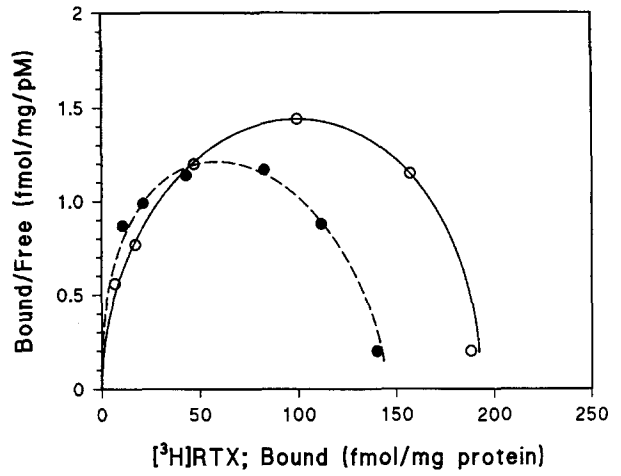

Fig. 4. Scatchards plots of specific [^{3}H]resiniferatoxin binding to pig dorsal horn of the spinal cord membranes in the absence (open circles) or presence (closed circles) of 20 μM trifluoperazine. Theoretical curves were created using binding parameters (K_d values are 67 pM and 61 pM, Hill coefficients are 2.1 and 1.9, and B_{max} values are 194 fmol/mg protein and 144 fmol/mg protein in the absence or presence of trifluoperazine, respectively) from a fit to the allosteric Hill equation. Data are from a representative experiment; 2 additional experiments gave similar results (see Table 1).

previous findings (Acs et al., 1995), rimcazole at a concentration of 100 μM inhibited [^{3}H]resiniferatoxin binding to rat spinal cord preparations (31 ± 6% of control binding; mean ± S.E.M.; $n = 4$; not shown) but not to human spinal cord preparations (103 ± 3% of control binding; mean ± S.E.M.; 3 independent determinations; not shown).

As expected, capsaicin affected the binding of 13 pM [^{3}H]resiniferatoxin in a bi-phasic fashion (Fig. 3) although the initial increase was consistently less than that produced by non-radioactive resiniferatoxin in a parallel experiment (compare with Fig. 1). In keeping with this, a computer fit to the modified Hill equation revealed a cooperativity index of 1.7 ± 0.1 (mean ± S.E.M.; 3 determinations) which is significantly less (Student's t-test, $P < 0.05$) than that determined in the presence of non-radioactive resiniferatoxin (2.2 ± 0.1, see above). Capsaicin displaced bound [^{3}H]resiniferatoxin from porcine dorsal horn membranes with an affinity of 3 ± 1 μM (mean ± S.E.M.; 5 determinations); in parallel experiments, capsazepine displayed a similar affinity (a K_i of 5 ± 3 μM; mean ± S.E.M.; 4 determinations; not shown). In keeping with this, a clearly visible though incomplete reduction by 10 μM capsaicin or capsazepine (47% and 39% decrease in the density of labelling, respectively; both significant at a P value of 0.001) was observed in the autoradiographic labelling by [^{3}H]resiniferatoxin of porcine spinal cord sections (Fig. 2). Unexpectedly, unlike non-radioactive resiniferatoxin or capsaicin, capsazepine did not enhance [^{3}H]resiniferatoxin binding to pig dorsal horn membranes (Fig. 3).

Scatchard analysis of [^{3}H]resiniferatoxin binding in the absence or presence of 20 μM trifluoperazine revealed a significant (Student's t-test, $P < 0.01$) decrease in B_{max} (from 181 ± 9 fmol/mg protein to 121 ± 5 fmol/mg protein; mean ± S.E.M.; 3 experiments) without a measurable change in affinity or cooperativity (Fig. 4 and Table 1). Thus, the resiniferatoxin binding inhibitory action of this drug must at least in part be non-competitive. However, the enhancement of [^{3}H]resiniferatoxin binding at lower concentrations of trifluoperazine (see above) cannot simply be due to a reduction in B_{max}, suggesting that low and high trifluoperazine concentrations have differential effects on resiniferatoxin binding. In parallel experiments, 10 μM capsaicin or a similar concentration of capsazepine increased the K_d of [^{3}H]resiniferatoxin binding from 61 ± 8 pM to 120 ± 11 pM or to 101 ± 7 pM, respectively (Table 1; mean ± S.E.M.; 3 experiments). Neither capsaicin nor capsazepine affected the maximal receptor density (Table 1).

Table 1
Parameters of [^{3}H]resiniferatoxin binding to porcine dorsal horn membranes in the absence or presence of trifluoperazine (20 μM), capsaicin (10 μM), or capsazepine (10 μM)

	K_d (pM)	Cooperativity index	B_{max} (fmol/mg protein)
Control	61 ± 8	2.0 ± 0.1	181 ± 9
Trifluoperazine	69 ± 10	1.9 ± 0.1	121 ± 5 [a]
Capsaicin	120 ± 11 [a]	1.7 ± 0.2	175 ± 12
Capsazepine	101 ± 7 [a]	2.1 ± 0.1	184 ± 11

Means ± S.E.M.; 3 determinations. [a] Student's t-test; $P < 0.01$.

4. Discussion

The interaction of neuroleptic and tricyclic antidepressant drugs at spinal vanilloid (capsaicin) receptors may provide a rational basis to understand their adjuvant analgesic action. At therapeutic concentrations (0.5–5.0 μM) (Connolly et al., 1992), the phenothiazine neuroleptic drug trifluoperazine enhances the specific binding of [^{3}H]resiniferatoxin, an ultrapotent capsaicin analog, to both human and rat spinal cord preparations (Acs et al., 1995). Vanilloid receptors bind agonists in a positive cooperative fashion (Szallasi et al., 1993a; Acs and Blumberg, 1994; Szallasi, 1994). This binding behaviour is generally believed to control receptor activity at critically low endogenous ligand concentrations (Maderspach and Fajszi, 1982; Schwartz and Skafar, 1993). If endogenous vanilloids exist, trifluoperazine may likewise amplify their binding to vanilloid receptors. A higher receptor occupancy by these endogenous ligands may potentiate cation, predominantly Ca^{2+}, influx through the connected cation channel which, in turn, is thought to impair the function of the affected neurons via ill-defined subcellular mechanisms (see Bevan et al., 1987; Holzer, 1991 for reviews). This theory is substantiated by the finding that 10 μM trifluoperazine is in fact able to enhance Ca^{2+} uptake induced by low (submicromolar) capsaicin concentrations (Acs et al., 1995).

The mechanism(s) by which neuroleptics bind to vanilloid receptors is yet to be clarified. Previously, 3 μM trifluoperazine was found to increase both affinity and cooperativity of resiniferatoxin binding to human vanilloid receptors whereas a higher (10 μM) concentration of trifluoperazine, conversely, decreased both binding parameters (Acs et al., 1995). Thus it is not unlikely that trifluoperazine binds to an allosteric modulation site distinct from the [^{3}H]resiniferatoxin binding sites thereby changing the cooperativity and affinity of agonist binding simultaneously. The present finding that trifluoperazine reduces the maximal receptor density in pig spinal cord is in accord with an allosteric interaction. Moreover, it implies further species-related differences in the allosteric modulation of spinal vanilloid receptors.

Whereas trifluoperazine affected [^{3}H]resiniferatoxin binding in a bi-phasic fashion in both human and rat spinal cord, rimcazole showed marked species-related differences: in the rat, it inhibited resiniferatoxin binding without a prior enhancement, whilst in man, conversely, it enhanced but did not inhibit resiniferatoxin binding (Acs et al., 1995). These findings imply that the rat is not a really suitable model to study to interaction of neuroleptics at vanilloid receptors. Here we show that rimcazole, like in human spinal cord, increases but does not inhibit [^{3}H]resiniferatoxin binding to pig dorsal horn preparations. Moreover, we confirm that rimcazole inhibits but does not enhance resiniferatoxin binding to rat spinal cord membranes. Receptors may bind ligands differently in intact tissues and in membrane homogenates (Davis et al., 1977). Rimcazole, however, does not inhibit autoradiographic labelling of intact vanilloid receptors in porcine spinal cord, either, arguing against an artifact of tissue homogenization.

An unexpected finding of the present study is the monophasic inhition of [^{3}H]resiniferatoxin binding by capsazepine, a vanilloid receptor antagonist (Bevan et al., 1992). Thus, capsazepine presumably binds to vanilloid receptors in a non-cooperative fashion in this system. Nonetheless, Scatchard analysis suggests that capsazepine inhibits resiniferatoxin binding in accord with a competitive mechanism both in the rat (Szallasi et al., 1993b) and the pig (this study). A possible explanation of the forementioned results is an 'allosteric competitive' inhibition, as exemplified by non-peptide inhibitors of tachykinin NK_1 receptors (Gether et al., 1994; Rosenkilde et al., 1994). Such inhibitors do not share the contact points recognized by the agonists, thus the affinity for agonists and allosteric competitive antagonists, respectively, may be regulated differentially by structural and/or conformational changes in the receptor protein (Rosenkilde et al., 1994; Elling et al., 1995). In keeping with this, reducing agents can, in fact, decrease the affinity of vanilloid receptors to resiniferatoxin (Szallasi et al., 1993b) and, conversely, increase the affinity to capsazepine (Szallasi, unpublished obervations).

We conclude that the neuroleptic drugs trifluoperazine and rimcazole modulate [^{3}H]resiniferatoxin binding to pig dorsal horn membranes (prepared 'freshly') in a fashion similar to that described in human dorsal horn preparations (obtained post-mortem). The binding behaviour of neuroleptics in human and porcine dorsal horn preparations, however, contrasts to that observed in the rat. Thus, the similar binding behaviour of trifluoperazine and rimcazole in human and porcine spinal cord preparations, which is at variance with their binding to rat spinal cord membranes, most likely reflect species-related differences in the mechanism(s) by which neuroleptic drugs interact at specific resiniferatoxin binding sites. Given the similarity in the binding of trifluoperazine and rimcazole to human and porcine dorsal horn preparations, porcine spinal cord, available from slaughter houses in unlimited quantities, appears to be a useful tool to further study the mechanisms by which neuroleptics interact at vanilloid receptors. In vivo capsaicin effects are well explored in the pig and pigs can easily be given drugs intrathecally. We think therefore that the pig may be a good model to evaluate the biological relevance of the binding of neuroleptics to vanilloid receptors.

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