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Evidence for release of glutamic acid, aspartic acid and substance P but not γ-aminobutyric acid from primary afferent fibres in rat spinal cord

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Abstract

In vitro superfusion release experiments and autoradiography were carried out on spinal cords of neonatally capsaicin-treated rats. Electrical and chemical stimulations significantly increased the release of aspartate, glutamate and γ-aminobutyric acid (GABA) from hemisected dorsal horn slices of vehicle-treated animals. In capsaicin-treated rats, the evoked release of aspartate, glutamate and substance P but not GABA, were significantly lower. Capsaicin (1 μM) stimulated the release of aspartate and glutamate, as reported for substance P, in control slices but this effect was not as apparent in tissues from capsaicin-treated rats. Evoked GABA release was not affected in either case. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, dizocilpine and $GABA_B$ binding sites were highly localised in the substantia gelatinosa. Capsaicin treatment did not affect the affinity of the binding sites in all four cases but significantly reduced the density of kainate, dizocilpine and $GABA_B$ binding sites. The data suggest that capsaicin-sensitive primary afferent fibres release aspartate, glutamate and Substance P following high-intensity stimulations and that this release might be modulated by presynaptic glutamate and $GABA_B$ receptors present on these terminals.

Keywords: Capsaicin treatment, neonatal; Superfusion; Autoradiography; Substance P; Glutamate receptor; $GABA_B$ receptor

1. Introduction

Aspartate, glutamate and γ-aminobutyric acid (GABA) are believed to play primary roles in the regulation of nociception (Salt and Hill, 1983; Sawynok, 1987). The involvement of the excitatory amino acids and $GABA_B$ receptors in nociception has been observed in various models of pain (Cutting and Jordan, 1975; Cahusac et al., 1984; Hammond and Drower, 1984; Aanonsen and Wilcox, 1987; Malcangio et al., 1991) and studies on rodent spinal cords have shown high densities of excitatory amino acid receptor binding sites, *N*-methyl-D-aspartate (NMDA), kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), in the superficial laminae of the dorsal horn (Greenamyre et al., 1984; Monaghan and Cotman, 1985; Jansen et al., 1990; Henley et al., 1993) where most nociceptive primary afferent fibres terminate (Cervero and Iggo, 1980; Brown, 1982). These observations parallel those reported for similar experiments carried out on human spinal cord (Jansen et al., 1990; Shaw et al., 1991; Chinnery et al., 1993; Shaw and Ince, 1994). Although immunohistochemical techniques have demonstrated that there is a high content of excitatory amino acids localised in vesicles (Broman and Ådahl, 1994) within the primaryafferent terminals (Westlund et al., 1989a, b), there have been few reports about the synaptic localisation of excitatory amino acid binding sites.

GABA appears to be present in the intrinsic interneurones of the spinal cord (Magoul et al., 1987; Todd and McKenzie, 1989) and exerts its actions via the $GABA_A$ and $GABA_B$ receptors (Hill and Bowery, 1981; Bowery et al., 1983). Autoradiographic studies using rat and human tissues have shown that, while the $GABA_A$ sites are evenly distributed throughout the laminae of the spinal cord, $GABA_B$ sites appear to be highly localised within the substantia gelatinosa (Price et al., 1984; Waldovogel et al., 1990; Malcangio et al., 1993). Neonatal capsaicin treatment and dorsal rhizotomy both reduce the density of $GABA_B$ sites in the rat substantia gelatinosa to the same extent (~ 40–50%; Price et al., 1984; Price et al., 1987) which suggests that about half of the $GABA_B$ sites de-

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Table 1
Conditions for $GABA_B$ and excitatory amino acid receptor binding site assays

Binding site	$GABA_B$	AMPA	Kainate	Dizocilpine
Ligand	50–200 μM [^{3}H]GABA	10–200 μM [^{3}H]AMPA	10–200 μM [^{3}H]kainate	5–40 μM [^{3}H]dizocilpine
Inhibitor	100 μM (-)baclofen	100 μM quisqualate	100 μM kainic acid	100 μM dizocilpine
Wash time	2 × 3 s	1 s, 10 s	1 s, 20 s	2 × 30 s
Wash temperature	23°C	23°C	4°C	4°C
Incubation time	20 min	40 min	60 min	30 min
Incubation temperature	23°C	23°C	23°C	23°C
Additional agents	40 μM isoguvacine 2.5 mM $CaCl_2$	100 mM KSCN 2.5 mM $CaCl_2$	None	10 μM glutamic acid 10 μM glycine 2.5 mM $CaCl_2$

tected are located on the small-diameter primary afferent nerve terminals.

In the present study, we have attempted to determine the neuronal origin and roles of aspartate, glutamate and GABA in the dorsal horn of the spinal cord by performing in vitro superfusion experiments on vehicle- and capsaicin-treated rats. Comparison with substance P-like immunoreactivity release was made under the same conditions. We also sought to ascertain the synaptic localisation of the glutamate and $GABA_B$ receptor binding sites in the spinal cord by using autoradiographic methods.

2. Materials and methods

Two-day-old Wistar rats (School of Pharmacy, London, UK), were randomly divided into two groups and injected (s.c.) with either 50 mg/kg capsaicin or an equal volume of vehicle (1 : 1 : 8 ethanol : Tween 80 : saline). Three months after the injection, these animals (200–300 g) were sacrificed and the lumbar enlargement of the spinal cords excised for either neurochemical or autoradiographic study.

2.1. In vitro release

Tissue slices were prepared as described previously (Malcangio and Bowery, 1993; Malcangio and Bowery, 1994). Briefly, segments of the lumbosacral cord (~ 2 cm) were removed and hemisected in aerated (95% O_2/5% CO_2) ice-cold Krebs'-bicarbonate solution to yield 200–300 μm thick dorsal horn slices. Each tissue slice was positioned in the central division of a three-compartment bath while the two pairs of intact dorsal roots were draped in the lateral divisions on bipolar platinum electrodes. The slices were constantly superfused at 1 ml/min with oxygenated Krebs'-bicarbonate solution of the following composition (mM): NaCl, 124; KCl, 4; KH_2PO_4, 1.2; $NaHCO_3$, 26; $MgSO_4$, 1.2; $CaCl_2$, 2.5 and glucose, 10. After an equilibration period of 60 min, consecutive 3-min samples were collected. For electrical stimulation, two 20-V, 0.5-ms stimuli at 1 Hz were applied for 3 min during collection of the fourth and eighth fractions. Chemical stimulation was performed with either a raised concentration of potassium (K^+) or veratridine. In the former case, while the fourth and eighth fractions were collected, the superfusion medium was substituted with one containing 50 mM KCl. Tonicity of the bathing solution was maintained by removal of the appropriate amount of NaCl. In the latter case, the fourth and eighth samples were collected when the tissue was bathed in a solution containing 50 μM veratridine. Superfusate samples were kept at −80°C until they were chemically analysed. Amino acid concentrations in the superfusate samples were determined by reversed-phase high-performance liquid chromatography (HPLC) coupled with *o*-phthaldialdehyde precolumn derivatisation and fluorimetric detection (Lindroth and Mopper, 1979). In all cases, the mean concentration of the individual amino acids in the first three samples was taken as the spontaneous outflow. Concentrations of amino acids in all subsequent fractions were expressed as a percentage of this

Table 2
Effect of 1 μM capsaicin, superfused through the system for 5 min, on the release of aspartate, glutamate and GABA from hemisected rat dorsal horn slices

Amino acid	Basal release (nM)		Capsaicin-evoked release (% basal release)	
	Controls (n = 9–12)	Neonatal capsaicin-treated (n = 9–12)	Controls (n = 5)	Neonatal capsaicin-treated (n = 5)
Aspartate	269.51 ± 29.1	246.51 ± 32.5	313.72 ± 26.0 [a]	139.48 ± 9.4
Glutamate	248.32 ± 14.9	225.51 ± 23.1	321.90 ± 19.0 [a]	132.08 ± 4.5
GABA	156.12 ± 21.6	148.73 ± 16.2	121.35 ± 8.3	105.61 ± 4.6

All data are expressed as mean ± S.E. [a] $P < 0.01$ when raw values were compared with basal outflow levels (Student's t test).

basal value. Collection of samples for substance P-like immunoreactivity release and its determination by RIA were performed exactly as reported previously (Malcangio and Bowery, 1993, 1994).

2.2. Autoradiography

The methods employed were based on those described previously (Malcangio et al., 1993). The lumbar enlargements were removed, frozen by immersion in iso-pentane cooled in liquid nitrogen and stored at −80°C. Ten micron transverse sections were cut, thaw-mounted on gelatine-coated slides and stored for up to 3 weeks. Frozen sections were brought to ambient temperature for 1 h before washing for 60 min in 50 mM Tris buffer, adjusted to pH = 7.4 at room temperature with either hydrochloric acid ($GABA_B$, dizocilpine and AMPA binding sites) or acetic acid (kainate binding sites). This process was repeated for another 20 min in fresh buffer. Binding in each case was initiated by covering the slices with 200 μl of the appropriate solution. In all the experiments, the binding reaction was terminated by aspiration of the labelled solution followed by two washes in buffer and then a short rapid immersion in deionised-distilled water to remove buffer salts. Table 1 summarizes the binding conditions followed. Autoradiograms were generated by apposition of dried Ilford K5 emulsion-coated coverslips to the sections-bearing slides (Young and Kuhar, 1979; Malcangio et al., 1993). These slide-coverslip composites were stored desiccated at room temperature in lightproof boxes for 12–15 days. After exposure, the emulsion was developed (Kodak D19), fixed (Unifix, Kodak) and the sections stained with 0.1% Cresyl violet (Sigma Chemicals, Poole, UK).

Autoradiographic silver grains on the coverslips were apparent under transmitted darkfield optics. The autoradiograms were analyzed using a Quantimet 970 Image Analyzer (Cambridge Research Instruments, UK). Total and nonspecific binding were quantified by counting the silver grains overlying the substantia gelatinosa regions of each spinal cord section. The amount of ligand bound (fmol/mg tissue), as represented by the number of grains per unit area, was determined by using [^{3}H]microscales (Amersham International, UK), which had been co-exposed and developed with the spinal cord sections. Three readings were taken for the substantia gelatinosa of each dorsal horn section. As there was no significant difference between the readings from both sides, the results were pooled and the mean of these values taken as the reading for that section. For each concentration, eight total and eight non-specific readings were taken from each rat. Specific binding of radioligands to the various binding sites was taken to be the difference between the total and nonspecific values. The average specific binding value for each concentration was then taken as a single value for statistical analysis (Student's t test).

Capsaicin (8-methyl-*N*-vanillyl-6-noneamide), Trizma base, Tween 80, glutamic acid (monosodium salt), glycine (aminoacetic acid, Ultragrade), quisqualic acid, kainic acid and potassium thiocyanate were from Sigma Chemicals. Dizocilpine hydrogen maleate was obtained from Research Biochemicals (UK). Tritiated AMPA (53.0 Ci/mmol), GABA (87.3 Ci/mmol), kainate (58.0 Ci/mmol) and dizocilpine (22.5 Ci/mmol) were purchased from New England Nuclear, Herts, UK. Isoguvacine · HCl and (−)-

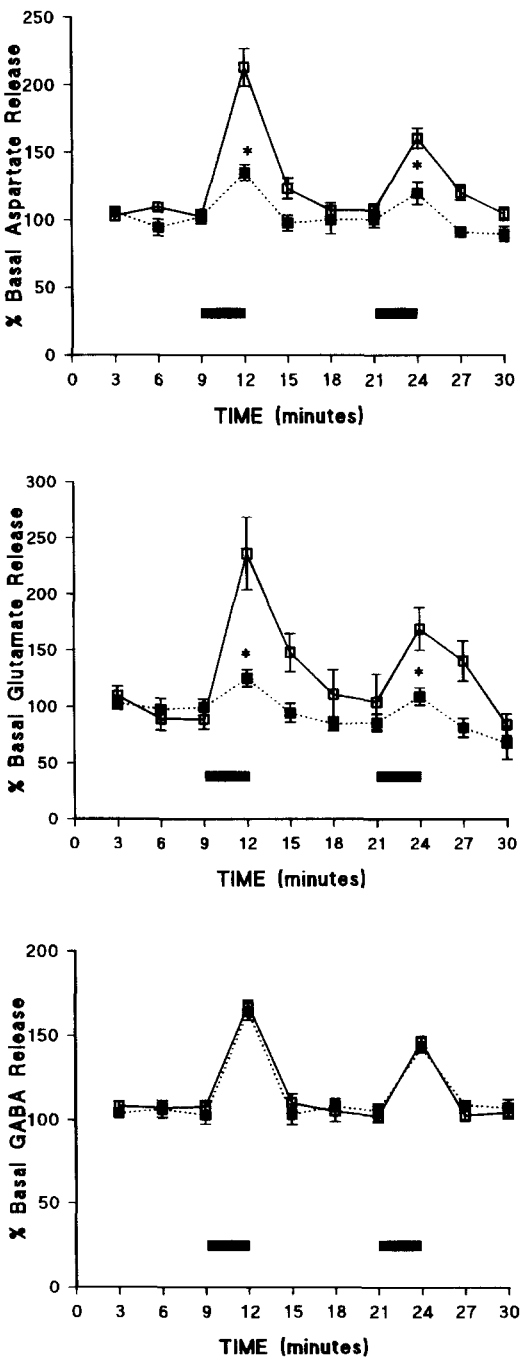

Fig. 1. Effect of electrical stimulation (black bars; 20 V, 1 Hz, 0.5 ms, 3 min) on the release of aspartate, glutamate and GABA from vehicle- (open symbols) and capsaicin-treated (solid symbols) animals. Results are expressed as mean % basal release ± S.E. for 3–6 animals. * $P < 0.05$ when raw data from capsaicin-treated animals were compared with those of vehicle control (Student's t test).

baclofen were from Cambridge Research Biochemicals (UK) and CIBA-Geigy (Basel, Switzerland), respectively.

3. Results

3.1. In vitro release

Basal release of the three amino acids measured in the superfusates from the spinal cords of vehicle- and capsaicin-treated animals were similar (Table 2). In vehicle-treated animals, electrical stimulation, high K^+ and veratridine increased the outflow of aspartate, glutamate and GABA (Figs. 1–3). The electrically and chemically stimulated release of aspartate and glutamate were lower in the capsaicin-treated rats. By comparison, GABA release evoked by the three stimuli was similar in both control and capsaicin-treated animals (Figs. 1–3). When capsaicin (1 μM) was added to the superfusing medium for 5 min, the

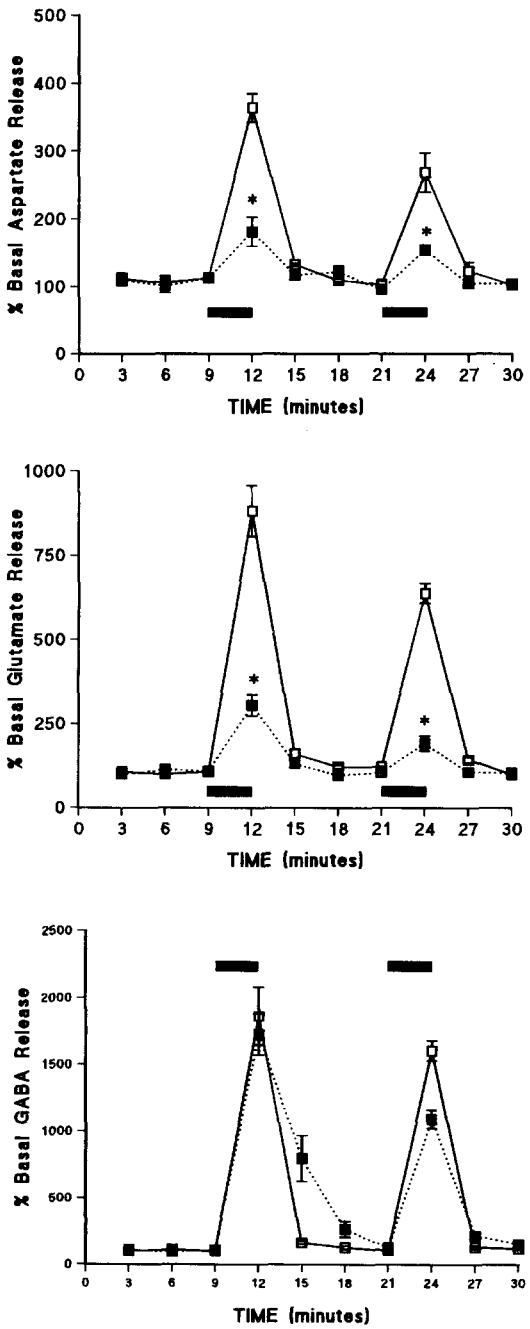

Fig. 2. Effect of elevated K^+ (black bars; 50 mM) on the release of aspartate, glutamate and GABA from vehicle- (open symbols) and capsaicin-treated (solid symbols) animals. Results are expressed as mean % basal release ±S.E. for 3 animals. * $P < 0.05$ when raw data from capsaicin-treated animals were compared with those of vehicle control (Student's t test).

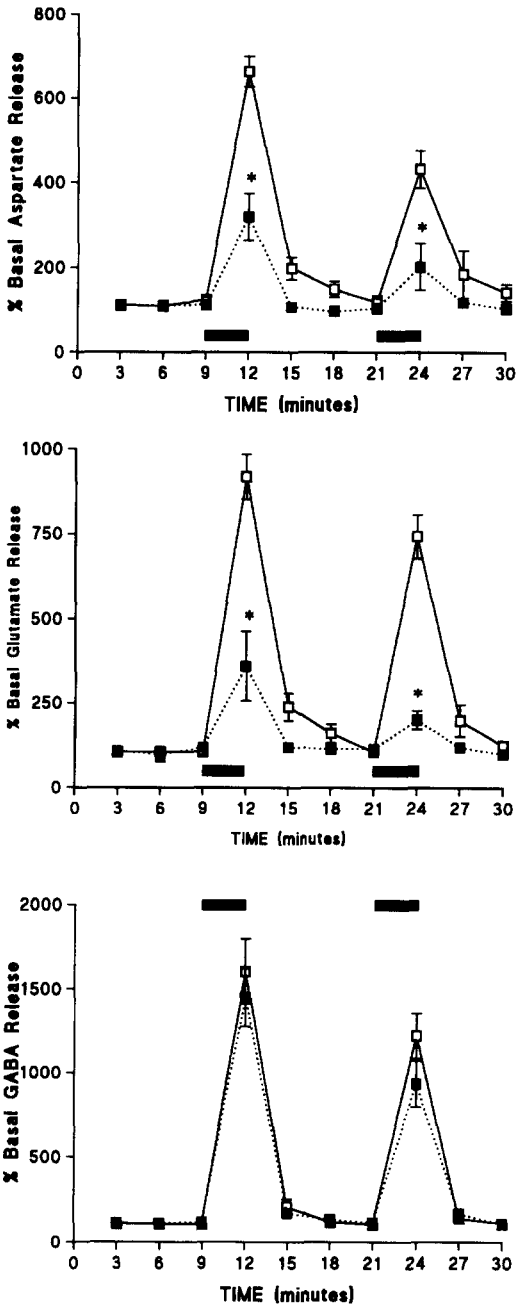

Fig. 3. Effect of veratridine (black bars; 50 μM) on the release of aspartate, glutamate and GABA from vehicle- (open symbols) and capsaicin-treated (solid symbols) animals. Results are expressed as mean % basal release ±S.E. for 3 animals. * $P < 0.05$ when raw data from capsaicin-treated animals were compared with those of vehicle control (Student's t test).

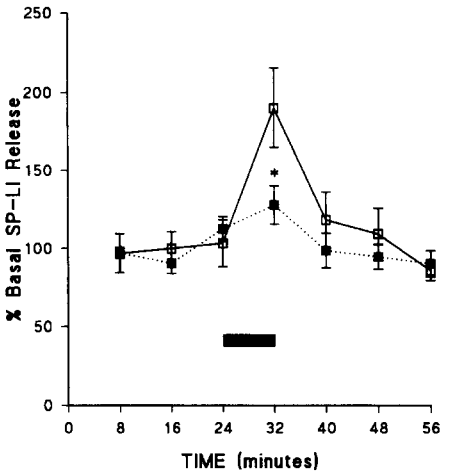

Fig. 4. Effect of electrical stimulation (black bar; 20 V, 1 Hz, 0.5 ms, 8 min) on substance P-like immunoreactivity release from vehicle- (open symbols; $n = 7$) and capsaicin-treated (solid symbols; $n = 5$) animals. Results are expressed as mean % basal release ±S.E. * $P < 0.05$ when raw data from capsaicin-treated animals were compared with those of vehicle control (Student's t test).

concentrations of both aspartate and glutamate in the superfusates collected from control spinal cords rose significantly above basal levels (Table 2). In contrast, capsaicin failed to evoke a significant increase in the release of glutamate and aspartate from cords in the capsaicin-treated group. Capsaicin had no significant influence on the release of GABA from vehicle- or capsaicin-treated animals (Table 2).

For comparison, the electrically evoked release of substance P-like immunoreactivity was examined in cords from 5 animals treated neonatally with capsaicin under the same conditions. The results from these experiments are shown in Fig. 4 together with data obtained from 7 vehicle-treated rats. As expected, capsaicin suppressed the evoked release by > 80% but had no effect on basal release.

3.2. Autoradiography

Binding of all four radioligands was concentration-dependent and occurred in all the laminae with the highest density for each ligand located in the substantia gelatinosa (Figs. 5 and 6). Specific binding to $GABA_B$, AMPA, kainate and dizocilpine receptor binding sites was > 85, 65, 60 and 65%, respectively. Scatchard analyses indicated that only a single binding site was present for all four ligands. The estimated K_d and B_{max} values obtained from saturation studies in this region are given in Table 3. Capsaicin treatment significantly reduced the density of $GABA_B$, kainate and dizocilpine binding sites by 46, 39 and 13%, respectively. In all cases, the K_d values were unaffected.

4. Discussion

Capsaicin is a selective excitant of polymodal C (Holzer, 1988) and A δ nociceptors (Szolcsanyi, 1985) and appears not to have any direct effect on other types of peripheral or central neurones (Jancsó et al., 1977; Holzer et al., 1980; Cervero and McRitchie, 1982). The neurotoxic action of capsaicin is dose-related (Jancsó and Kiraly, 1981) such that even small myelinated fibres can be similarly affected at higher doses (Jancsó et al., 1977; Lawson and Nickels, 1980; Nagy et al., 1983). Nevertheless, this model is still useful for studying the anatomical distribution and function of small-diameter primary afferents (e.g. Zhuo and Gebhart, 1994; Skilling and Larson, 1993; Meller et al., 1992; Kashiba et al., 1990).

The three stimuli used in this study act via different mechanisms. Veratridine evokes neurotransmitter release by directly opening sodium channels on neuronal cells (Narahashi, 1974) while high levels of extracellular K^+ depolarise the neurones and affect glial cells too. Electrical stimulation can be selectively manipulated to activate particular neurones or pathways. The parameters used in our studies were selected to activate the fast-conducting myelinated (A β) fibres as well as the slower conducting myelinated (A δ) and unmyelinated (C) fibres (Kangrga et al., 1990; Kangrga and Randić, 1991).

The electrically enhanced release of the three amino acids in this model has been shown in naïve rat spinal cords to be tetrodotoxin-sensitive and calcium-dependent (Teoh et al., 1995). These, therefore, indicate that a proportion of the excitatory amino acids measured arise from an excitable source via exocytosis. In control slices, elevated K^+ and veratridine also significantly increased the release of these amino acids above basal levels supporting the concept that these amino acids originate from a neuronal source. The stimulated responses for the excitatory amino acids were dramatically lower for the capsaicin-treated rats regardless of the stimulus applied. On the other hand, there was little difference in the stimulated responses for GABA between the two groups of animals. If the source of released glutamate and aspartate was not the primary afferent fibre, we would have expected to see no reduction in the K^+ or veratridine-mediated release in the capsaicin-treated rats.

Thus, our data suggest that the capsaicin-sensitive primary afferent fibres may be a potentially important source for the release of aspartate and glutamate during noxious stimulation. This concurs with the in vitro studies by Jeftinija et al. (1991), Kangrga and Randić (1990, 1991) as well as in vivo studies by Skilling and Larson (1993) but contradicts that by Donnerer (1991) who suggested that the excitatory amino acids arise from capsaicin-insensitive nerve structures in the rat dorsal spinal cord. At the spinal level, both aspartate and glutamate are neurotransmitter candidates for neurones other than the primary afferents

(Rustioni and Cuenod, 1982; Potashner and Dymzyk, 1986). Both excitatory amino acids can also be released from glial elements by a calcium-independent mechanism when the extracellular concentration of K^+ is raised (Szatkowski et al., 1990). Hence, the role played by these nonprimary afferent cell types in the release of excitatory amino acids by K^+ and veratridine stimulation must be taken into account in Donnerer's preparation since Jeftinija et al. (1991) demonstrated that the glial cells in their preparation made no difference to the overall results. The

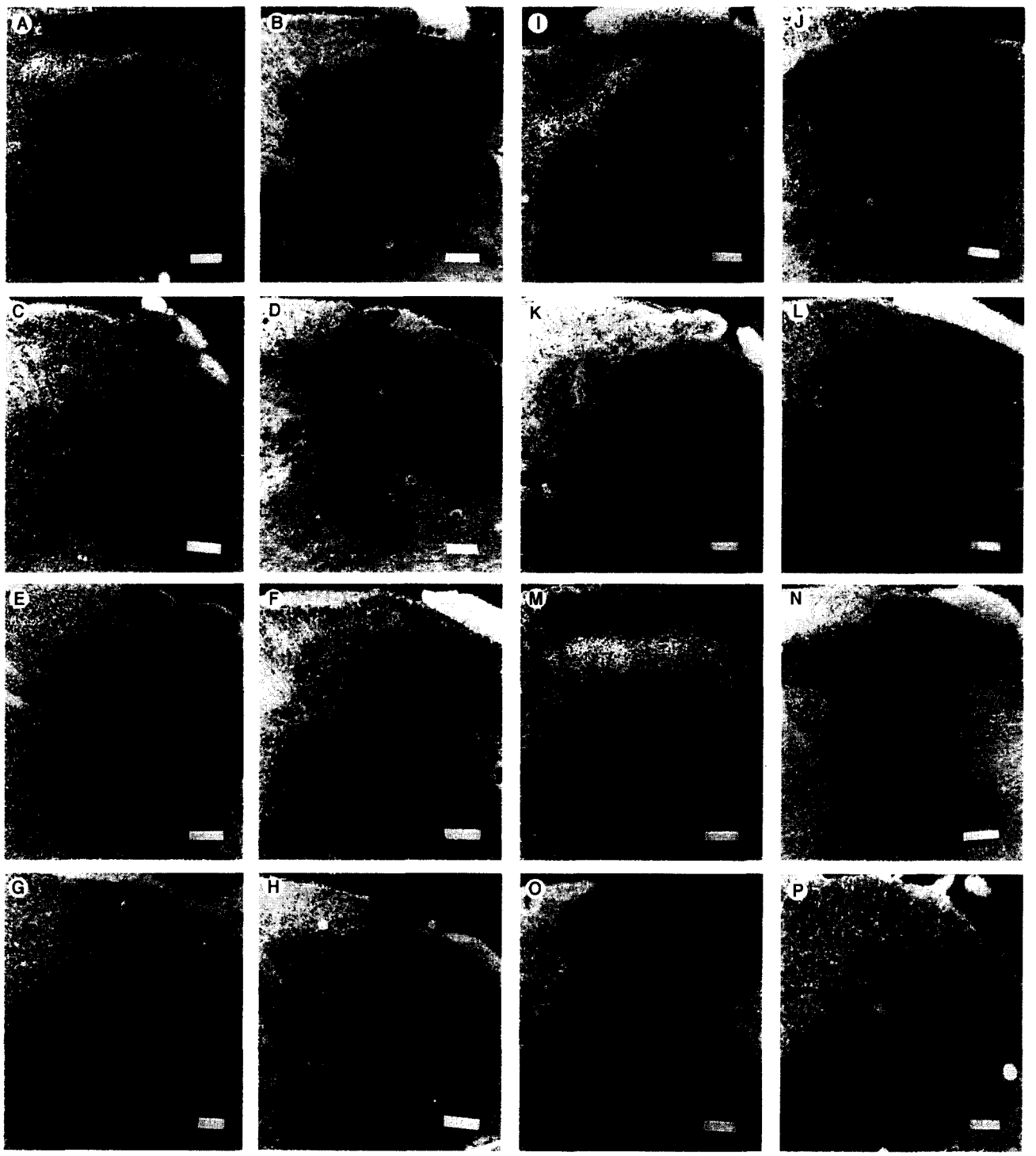

Fig. 5. Darkfield photomicrographs showing the right halves of rat dorsal horn spinal cord sections from vehicle- (A, B, E, F, I, J, M and N) and capsaicin-treated (C, D, G, H, K, L, O and P) rats. The density of the silver grains correspond positively to the population of receptor binding sites. Photomicrographs A, C, E, G, I, K, M and O depict the total and B, D, F, H, J, L, N and P, the nonspecific patterns of the highest concentrations of [^{3}H]GABA (A–D), [^{3}H]AMPA (E–H), [^{3}H]kainate (I–L) and [^{3}H]dizocilpine (M–P). Scale bar represents 200 μm.

Table 3
K_d and B_{max} values for $GABA_B$ and excitatory amino acid receptor-binding in the substantia gelatinosa of vehicle- and capsaicin-treated rats

Ligand	K_d (nM)		B_{max} (fmol/mg)	
	Vehicle	Capsaicin	Vehicle	Capsaicin
[^{3}H]GABA	164.0±30.5	158.9±49.4	256.0±13.9	137.9±11.6 [a]
[^{3}H]AMPA	44.6±16.1	41.4±14.1	315.5±27.4	262.1±22.0
[^{3}H]Kainate	21.1± 3.9	18.7± 4.9	637.4±26.0	387.3±16.5 [a]
[^{3}H]Dizocilpine	8.3± 2.8	6.7± 0.2	170.8± 4.9	148.9± 0.3 [a]

All data are expressed as mean±S.E. ($n=4$ rats for each group). Statistical difference between the two groups was assessed with the Student's t test. [a] $P < 0.05$.

difference between our veratridine results and those of Donnerer (1991) could be due to a difference in the preparations used. While Donnerer used transversely chopped dorsal horn slice microprisms, we believe that our longitudinal dorsal horn slices with intact dorsal roots represent the in situ condition more closely since the integrity of the rostra–caudally directed synaptic connections were undisrupted.

Jeftinija et al. (1991) and Kangrga and Randić (1991) have also showed that direct stimulation of the dorsal root ganglia cells with capsaicin significantly elevates the levels of aspartate and glutamate in the samples supporting the concept that some excitatory amino acid-containing neurones are capsaicin-sensitive. We observed a similar phenomenon on superfusing spinal slices with 1 μM capsaicin for 5 min. However, in our model, the possibility that a proportion of this enhanced release arises from noncutaneous sensory neuronal cell types cannot be dismissed since the whole tissue slice was exposed to the neurotoxin. Donnerer (1991), on the other hand, failed to evoke any release of the excitatory amino acids with

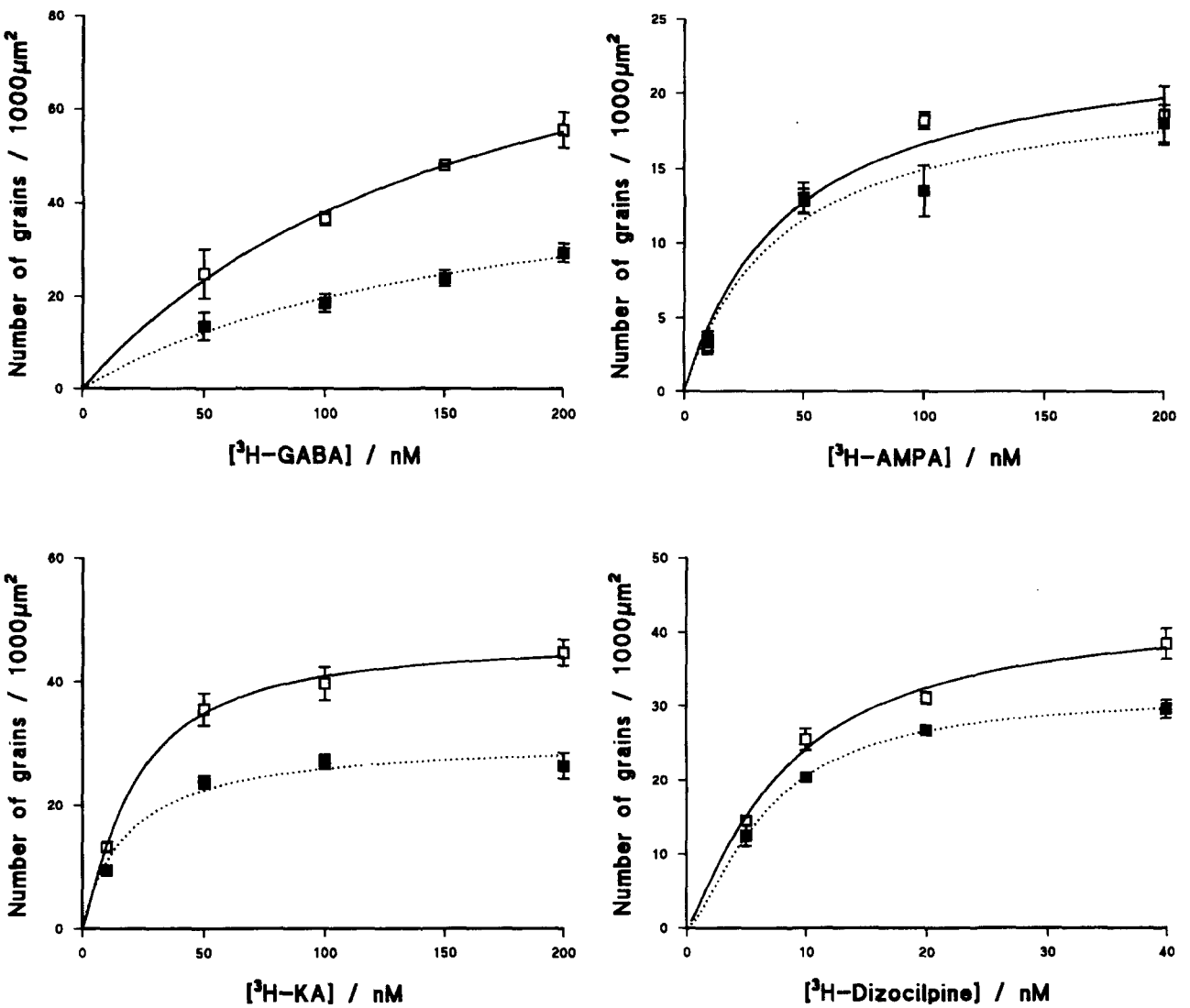

Fig. 6. Saturation curves for the binding of [^{3}H]GABA, [^{3}H]AMPA, [^{3}H]kainate and [^{3}H]dizocilpine to, respectively, $GABA_B$, AMPA, kainate and dizocilpine binding sites in the substantia gelatinosa. Open and solid symbols, respectively, denote data from animals treated neonatally with vehicle and 50 mg/kg capsaicin. Results are expressed as mean ± S.E. for 4 animals.

capsaicin. The reduction in stimulated excitatory amino acids release we observed in the capsaicin-treated rats concurs with data from in vivo studies on rats similarly treated (Skilling and Larson, 1993). Based on rhizotomy experiments, these workers also suggested that an important source of aspartate and glutamate is the intrinsic interneurones and that neonatal capsaicin treatment might interfere with the normal development of excitatory amino acid interneurones innervated by the substance P primary afferent C-fibres. While our results do not disprove this theory, it seems likely that since treatment with the neurotoxin caused a substantial fall in the stimulated release, that a significant proportion of the excitatory amino acids and substance P-like immunoreactivity measured in the superfusate samples in our preparation originate from the capsaicin-sensitive small-diameter primary afferents.

Our results complement previously reported functional (Kangrga and Randić, 1990, 1991; Giovannini et al., 1991; Jeftinija et al., 1991; Skilling and Larson, 1993) and anatomical (Battaglia and Rustioni, 1988; De Biasi and Rustioni, 1988; Westlund et al., 1989a, b) studies with regards to the role of the excitatory amino acids and substance P in the transmission of cutaneous information. However, our data only support the involvement of C-fibres in the release of aspartate and glutamate and do not indicate what proportion of the excitatory amino acids detected derive from other neuronal cell types. In accordance with the observations of Donnerer (1991) and Todd and McKenzie (1989), our data suggest that the increased release of GABA observed originates from the intrinsic interneurones. Hence, GABA probably plays a modulatory role at the spinal level by regulating the release of various neurotransmitters (Kangrga et al., 1991; Malcangio and Bowery, 1993, 1994) released from both the primary afferents and the intrinsic interneurones.

The distribution of $GABA_B$ receptor binding sites we observed concur with those previously reported (Price et al., 1984; Malcangio et al., 1993). The reduction in the number of $GABA_B$ receptor binding sites in our capsaicin-treated animals also mirror earlier observations (Price et al., 1984). Since there was no significant difference in the K_d values between the two groups of animals, our study further indicates that the decreased binding observed in rats treated neonatally with capsaicin is due to a reduction in the number of $GABA_B$ receptor binding sites and not a change in the binding affinity of the receptors. In agreement with earlier studies on rodent and human spinal cords (Greenamyre et al., 1984; Monaghan and Cotman, 1985; Jansen et al., 1990; Shaw et al., 1991; Chinnery et al., 1993; Henley et al., 1993; Shaw and Ince, 1994), we have demonstrated that the excitatory amino acid receptor binding sites are highly localised in the superficial laminae of the spinal cord. In our hands, capsaicin treatment reduced the degree of binding at the NMDA as well as the non-NMDA sites. This difference was also due to an alteration in the density of the binding sites (B_{max}) and not the affinity (K_d) of the receptors. However, only the decrease in dizocilpine and kainate binding sites reached statistical significance. These data, therefore, support previous suggestions that at least a proportion of the spinal excitatory amino acid receptors are located presynaptically on primary afferent fibres (Davies and Watkins, 1983; Agrawal and Evans, 1986; Liu et al., 1994). In the present study, dizocilpine and kainate binding sites were affected to different extents by neonatal capsaicin treatment. This suggests that, while both types of receptors are present on capsaicin-sensitive terminals, they may be found in different proportions on presynaptic and postsynaptic sites within the dorsal horn of the rat spinal cord.

A high density of excitatory amino acid and $GABA_B$ receptors in the substantia gelatinosa of the spinal cord complements the concept that excitatory amino acids and GABA are primary neurotransmitter candidates of the dorsal horn and suggest that these receptors are important in the mediation and/or modulation of peripheral sensory information. The highest density of these receptor binding sites are located in the substantia gelatinosa where the majority of the nociceptive primary afferent terminals are found (Cervero and Iggo, 1980; Brown, 1982). On the basis of our data, it would not be unreasonable to suggest that the release of excitatory amino acids from capsaicin-sensitive primary afferents following noxious stimulation is modulated not only by GABA from the intrinsic interneurones at presynaptic $GABA_B$ receptors but also by the excitatory amino acids themselves at the NMDA and kainate receptors located on the afferent terminals.

In conclusion, our study provides further evidence to support the idea that glutamate, aspartate and substance P, but not GABA, are released from the small-diameter capsaicin-sensitive primary afferent fibres following noxious stimulations. Our data also suggests that this release is to a certain extent, under the control of the presynaptic excitatory amino acid and $GABA_B$ receptors which appear to be present on capsaicin-sensitive primary afferent fibres.

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