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Respiratory actions of tachykinins in the nucleus of the solitary tract: effect of neonatal capsaicin pretreatment

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1 The respiratory response to microinjection of capsaicin and tachykinin receptor agonists into the commissural nucleus of the solitary tract (cNTS) was investigated in adult, urethane-anaesthetized rats which had been pretreated with capsaicin (50 mg kg^{-1} s.c.) or vehicle (10% Tween 80, 10% ethanol in saline) as day 2 neonates.

2 Microinjection of capsaicin (1 nmol) into the cNTS of vehicle-pretreated rats, significantly reduced respiratory frequency (59 breaths min^{-1}, preinjection control, 106 breaths min^{-1}) without affecting tidal volume (VT). In capsaicin-pretreated rats, the capsaicin-induced bradypnoea was markedly attenuated (minimum frequency, 88 breaths min^{-1}; control, 106 breaths min^{-1}).

3 In vehicle-pretreated rats, microinjection of substance P (SP, 33 pmol), neurokinin A (NKA, 33 pmol) and NKB (330 pmol), and the selective NK_1 tachykinin receptor agonists, [Sar^9,$Met(O_2)^{11}$]-SP (33 pmol) and septide (10 pmol), increased VT (maxima, 3.60–3.93 ml kg^{-1}) compared with preinjection control (2.82 ml kg^{-1}), without affecting frequency. The selective NK_3 agonist senktide (10 pmol) also increased VT (3.93 ml kg^{-1}) which was accompanied by a bradypnoea (−25 breaths min^{-1}). The selective NK_2 agonist, [Nle^{10}]-NKA(4-10) (330 pmol) increased VT slightly but significantly decreased frequency (−12 breaths min^{-1}). In capsaicin-pretreated rats, VT responses to SP and [Sar^9,$Met(O_2)^{11}$]-SP were increased whereas the response to septide was abolished. Both the VT and bradypnoeic responses to senktide and [Nle^{10}]-NKA(4-10) were significantly enhanced.

4 These results show that neonatal capsaicin administration markedly reduces the respiratory response to microinjection of capsaicin into the cNTS. The destruction of capsaicin-sensitive afferents appears to sensitize the NTS to SP, NKB, [Sar^9,$Met(O_2)^{11}$]-SP, senktide and [Nle^{10}]-NKA(4-10). Moreover, the loss of septide responsiveness in capsaicin-pretreated rats, suggests that 'septide-sensitive' NK_1 receptors may be located on the central terminals of afferent neurons.

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Abbreviations: cNTS, commissural nucleus of the solitary tract; f, frequency; NK, neurokinin; SP, substance P; VE, minute ventilation; VT, tidal volume

Introduction

The nucleus of the solitary tract (NTS) is the primary integration site for many visceral reflexes and is rich in the tachykinins substance P (SP), neurokinin A (NKA) and NKB (Douglas *et al*., 1982; Kalia *et al*., 1984; Jordan & Spyer, 1986; Nagashima *et al*., 1989). Our recent functional studies employing SP, NKA and NKB, receptor-selective agonists and nonpeptide antagonists suggest that all three tachykinin receptors (NK_1, NK_2 and NK_3) are present in the NTS and play a role in respiratory control at the brain stem level (Mazzone & Geraghty, 1999a; 2000). Microinjection of NK_1 receptor agonists into the commissural NTS (cNTS) increases tidal volume (VT) whereas NK_2 receptor agonists produce a bradypnoea (Mazzone & Geraghty, 2000). NK_3 receptor agonists cause a mixed response (increase VT and decrease frequency). Furthermore, tachykinin-induced increases in VT are attenuated by selective NK_1 and NK_3 receptor antagonists (RP 67580 and SR 142801, respectively) but not by the NK_2 receptor antagonist, SR 48968. However, the VT response to NKA is potentiated by SR 48968, suggesting that NK_2 receptors in the NTS modify respiration.

The vanilloid capsaicin, releases neurotransmitters including calcitonin gene-related peptide (CGRP), excitatory amino acids and tachykinins from the central and peripheral terminals of unmyelinated C-fibres and lightly myelinated Aδ-fibres which include peripheral chemoreceptor, baroreceptor and pulmonary afferents (Szolcsanyi, 1993). At the supraspinal level, vanilloid receptors (VR1) have been localized autoradiographically (using [^{3}H]-resiniferatoxin binding) and immunocytochemically to discrete regions of the brain stem, namely the NTS, area postrema and parts of the spinal trigeminal nucleus (Szallasi *et al*., 1995; Tominaga *et al*., 1998; Guo *et al*., 1999). We have previously shown that microinjection of capsaicin into the cNTS produces a dose-dependent bradypnoea which is blocked by SR 48968 and SR 142801 but not by RP 67580 (Mazzone & Geraghty, 1999a). Thus, the capsaicin-induced respiratory slowing is at least partly mediated by the release of tachykinins and their subsequent stimulation of NK_3 and possibly NK_2 receptors.

Systemic administration of capsaicin to neonatal rats destroys the majority of C- and Aδ-fibres (Szolcsányi, 1993). The SP content of the NTS is markedly reduced in capsaicinized rats compared with vehicle-pretreated controls suggesting the loss of tachykinin-containing afferents (Helke & Eskay, 1985; Takano *et al*., 1988). Moreover, the respiratory response to hypoxia (increased ventilation) is reduced by 40% in adult rats pretreated with capsaicin as neonates (De Sanctis *et al*., 1991). The aim of the present study was to determine

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whether neonatal capsaicin pretreatment alters the adult respiratory response to microinjection of capsaicin and tachykinins into the cNTS.

Methods

Capsaicin pretreatment

All experimental procedures were approved by the University of Tasmania Ethics Committee (Animal Experimentation; project 98010). Neonatal (P2) Hooded Wistar rat pups were placed in an ice bath until neck muscle tone was lost. Pups were subsequently treated with 50 mg kg^{-1} s.c. capsaicin or vehicle (10% ethanol, 10% Tween 80 in normal saline) and revived under an infrared lamp. Both groups of rats were allowed to mature for 10 weeks and were of similar weight at the time of experimentation (vehicle-pretreated, 277 ± 9 g, $n = 10$; capsaicin-pretreated, 268 ± 12 g, $n = 10$).

Surgery

Mature vehicle- and capsaicin-pretreated rats were anaesthetized using urethane (0.5 g kg^{-1} i.p. and 0.5 g kg^{-1} s.c.) and prepared for microinjection studies as described previously by our laboratory (Mazzone & Geraghty, 1999a,b; 2000). Briefly, rats were mounted in a Kopf stereotaxic apparatus and the dorsal aspect of the brain stem was exposed by a craniotomy. Respiration, which was spontaneous and rhythmic, was recorded using subcutaneous electrodes and a calibrated impedance converter (UFI, Morro Bay, California, U.S.A.).

Injection of agents into the cNTS

For capsaicin injections, a glass micropipette was mounted in a micromanipulator and using obex as a reference point, the needle was inserted into the cNTS: AP -15 to -15.3 mm; L 0–0.3 mm relative to bregma and 0.4 mm into the dorsal surface of the brain stem (Paxinos & Watson, 1986). Capsaicin (1 nmol in 500 nl of 25% ethanol in normal saline) was then injected over 30 s into the cNTS (dose based on previous study; Mazzone & Geraghty, 1999a). Respiratory movements were recorded for 30 min following the injection.

Tachykinin peptides and analogues were injected using a microprocessor-controlled pump (UltraMicroPump II, World Precision Instruments) mounted on a micromanipulator. All peptides were injected in 100 nl over 30 s. Ten vehicle- and capsaicin-pretreated rats were injected with three-to-four of the following agents: SP (33 pmol), NKA (10 pmol), NKB (100 pmol), [Sar^9,$Met(O_2)^{11}$]-SP (33 pmol), septide (1 pmol), senktide (1 pmol), [Nle^{10}]-NKA(4-10) (330 pmol) and vehicle (25% ethanol in saline). Except for [Nle^{10}]-NKA(4-10), which does not affect tidal volume (VT), doses were based on the approximate ED_{50} for increasing VT (Mazzone & Geraghty, 2000). Experiments were paired (one vehicle- and one capsaicin-pretreated rat) and the order of injection of agents was randomized for the ten pairs of rats. Rats were allowed to recover for 70 min between injections.

At the end of each experiment, rats were killed with an overdose of pentobarbitone, the brain stems were removed and later sectioned and stained to ascertain the exact injection site. Prior to removal of the brain stem, a polyethylene tube attached to a 5 ml syringe was inserted into the trachea and the impedance converter was calibrated by graded inflation of the lung. Respiratory movements were measured over a 20 s time interval and then converted to frequency, VT and minute ventilation (VE). Volumes were subsequently normalized to body weight. Data obtained from animals which showed evidence of the needle tract in the cNTS were compared using the Student *t*-test. $P < 0.05$ was considered statistically significant.

Drugs and materials

All tachykinin peptides and analogues (>95% purity) were purchased from Auspep (Melbourne, Australia). Capsaicin (>99% purity) was purchased from the Sigma Chemical Company. All agents for microinjection were dissolved in 25% ethanol in normal saline and stored in frozen aliquots. Other reagents were of analytical grade.

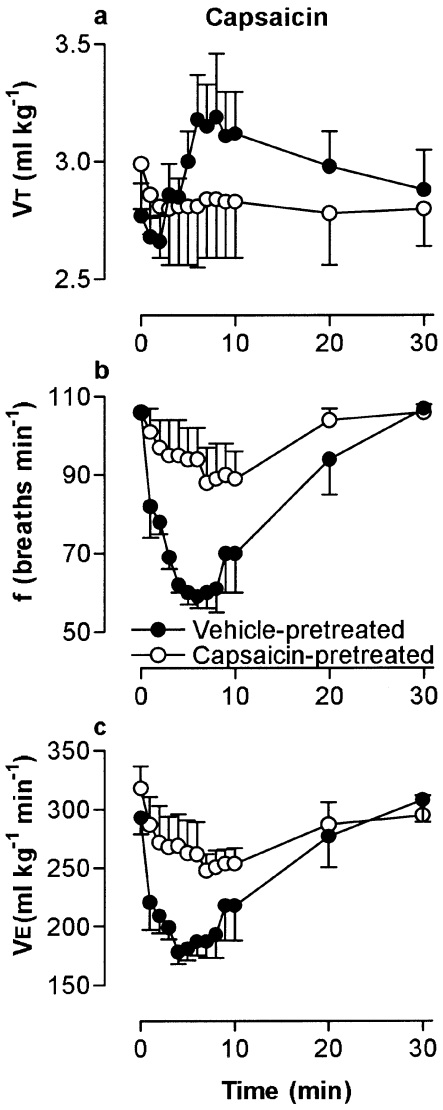

Figure 1 Respiratory response to microinjection of capsaicin (1 nmol) into the commissural nucleus of the solitary tract of urethane-anaesthetized, spontaneously breathing adult rats (10 weeks) pretreated as neonates (day 2) with capsaicin (50 mg kg^{-1} s.c.). (a) Tidal volume (VT), (b) respiratory frequency (f) and (c) minute ventilation (VE). Values are the mean of four animals and vertical bars show s.e.mean. Where error bars are not obvious, they are within the symbol.

Results

Effect of neonatal capsaicin-pretreatment on the respiratory response to capsaicin

In adult rats, pretreated with vehicle as day 2 neonates, microinjection of capsaicin (1 nmol) into the cNTS significantly slowed respiration (59±3 breaths min^{-1} compared with pre-injection control, 106±1 breaths min^{-1}; Figure 1a). VT was not altered by capsaicin and thus, VE followed a similar course to frequency (Figure 1b,c). Neonatal capsaicin-pretreatment did not affect baseline respiration but dramatically reduced the bradypnoeic response to capsaicin (minimum frequency, 88±9 breaths min^{-1}).

Table 1 Maximum changes in tidal volume, frequency and minute ventilation following microinjection of tachykinins into the cNTS of adult rats pretreated as neonates with vehicle or capsaicin

Peptide	*Dose* (pmol)	ΔVT (ml kg^{-1}) *VEH*	*CAP*	Δf (breaths min^{-1}) *VEH*	*CAP*	ΔVE (ml kg^{-1} min^{-1}) *VEH*	*CAP*
Vehicle	–	0.09±0.02	0.09±0.05	−4±2	−1±1	9±2	13±6
SP	33	0.94±0.06	1.29±0.25	−2±1	−6±5	99±6	126±32
NKA	10	0.83±0.10	1.07±0.21	−1±1	−1±1	102±12	99±25
NKB	100	0.87±0.07	1.97±0.09**	−2±1	−7±2	93±6	200±18**
Sar-SP	33	1.09±0.06	2.81±0.14**	−7±2	−7±3	92±11	252±39**
Septide	1	1.15±0.10	0.20±0.15**	−5±3	0±1	106±10	24±14**
Nle-NKA	330	0.19±0.03	0.46±0.07*	−16±1	−50±3**	−41±3	−129±9**
Senktide	1	1.19±0.05	3.96±0.08**	−23±3	−36±3*	44±10	161±26*

Data are the mean±s.e.mean of four experiments. ΔVT, Δf and ΔVE, maximum change in tidal volume, respiratory frequency and minute ventilation, respectively; VEH, vehicle pretreated; CAP, capsaicin-pretreated; SP, Substance P; NKA, neurokinin A; Sar-SP, [Sar^9,$Met(O_2)^{11}$]-SP; Nle-NKA, [Nle^{10}]-NKA(4–10). *$P<0.05$ and **$P<0.01$, significantly different from vehicle-pretreated rats (*t*-test).

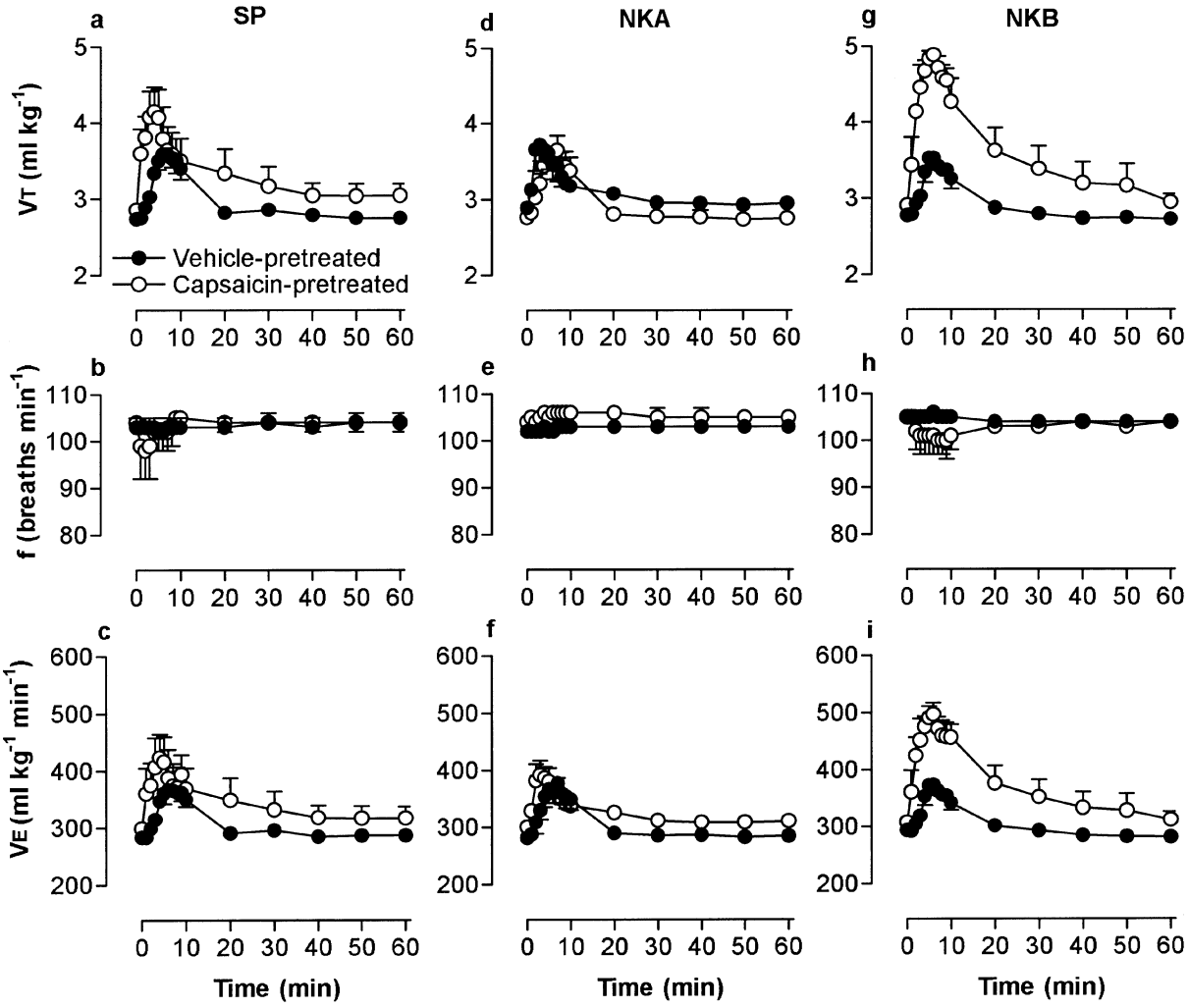

Figure 2 Respiratory response to microinjection of tachykinins into the commissural nucleus of the solitary tract of urethane-anaesthetized, spontaneously breathing adult rats (10 weeks) pretreated as neonates (day 2) with capsaicin (50 mg kg^{-1} s.c.). Doses injected were 33 pmol SP (a–c), 10 pmol NKA (d–f) and 100 pmol NKB (g–i). Tidal volume (VT), respiratory frequency (f) and minute ventilation (VE). Values are the mean of four animals and vertical bars show s.e.mean. Where error bars are not obvious, they are within the symbol.

Effect of neonatal capsaicin-pretreatment on the respiratory response to SP, NKA and NKB

The respiratory response to microinjection of the peptide vehicle (100 nl, 25% ethanol in normal saline) was negligible in both vehicle- and capsaicin-pretreated rats (Table 1). Microinjection of SP (33 pmol), NKA (10 pmol) and NKB (100 pmol) into the cNTS of vehicle-pretreated rats produced a significant increase in VT, with only minimal effects on frequency (Figure 2; Table 1). Thus, the VE response to agonists essentially followed the same course as the VT response (Figure 2c,f,i). In capsaicin-pretreated rats, microinjection of SP into the cNTS produced a significantly ($P<0.05$) greater VT response between 1 and 4 min after injection, although the maximum response was only slightly larger (but not significant) compared to vehicle-pretreated controls (Figure 2a; Table 1). In contrast, the VT response to microinjection of NKA was unchanged (Figure 2d). The increase in VT following injection of NKB was significantly greater in capsaicin-pretreated rats compared to vehicle-pretreated controls (Figure 2g; Table 1). Capsaicin-pretreatment did not alter the frequency response to any of the agonists (Figure 2b,e,h).

Effect of neonatal capsaicin-pretreatment on the respiratory response to receptor-selective tachykinins

In vehicle-pretreated rats, microinjection of the selective NK_1 agonists, [Sar^9,$Met(O_2)^{11}$]-SP (33 pmol) and septide (1 pmol), increased VT without affecting frequency (Figure 3a,b,d,e). Hence, VE followed a similar course to VT (Figure 3c,f). In capsaicin-pretreated rats, the response to [Sar^9,$Met(O_2)^{11}$]-SP was significantly ($P<0.01$) increased whereas the response to septide was markedly attenuated (Table 1). In the vehicle group, the NK_3 agonist, senktide (1 pmol), increased VT and also decreased frequency (Figure 3g,h). Both the VT and frequency responses to senktide were significantly increased in capsaicin-pretreated rats. However, stimulation of VT pre-

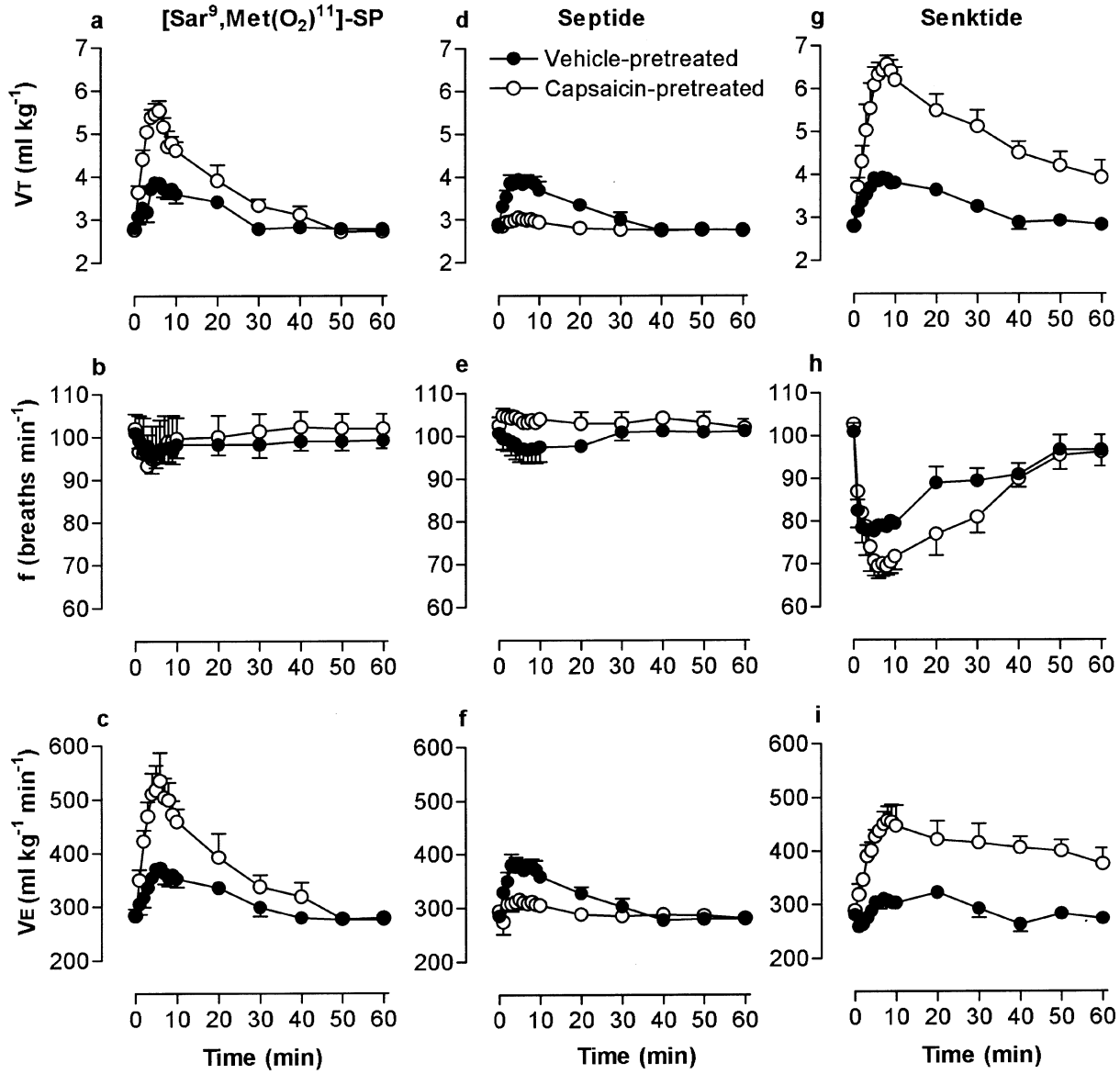

Figure 3 Respiratory response to microinjection of NK_1 and NK_3 receptor-selective tachykinins into the commissural nucleus of the solitary tract of urethane-anaesthetized, spontaneously breathing adult rats (10 weeks) pretreated as neonates (day 2) with capsaicin (50 mg kg^{-1} s.c.). Doses injected were 33 pmol [Sar^9,$Met(O_2)^{11}$]-SP (a–c), 10 pmol septide (d–f) and 10 pmol senktide (g–i). Tidal volume (VT), respiratory frequency (f) and minute ventilation (VE). Values are the mean of four animals and vertical bars show s.e.mean. Where error bars are not obvious, they are within the symbol.

dominated over the bradypnoea and thus, microinjection of senktide resulted in an overall increase in VE in both pretreatment groups (Figure 3i).

Microinjection of 330 pmol [Nle^{10}]-NKA(4-10) produced a bradypnoea which was markedly and significantly enhanced in capsaicin-pretreated rats (Figure 4b). Interestingly, [Nle^{10}]-NKA(4-10) had no effect on VT in vehicle-pretreated rats but significantly increased VT in the capsaicin-pretreated group (Figure 4a; Table 1).

Discussion

Our preceding study showed that all three tachykinin receptors (NK_1, NK_2 and NK_3) are present in the NTS, and undoubtedly play a role in the central control of respiration (Mazzone & Geraghty, 2000). The aim of the present study was to determine whether neonatal capsaicin administration, which destroys a large proportion of tachykinin-containing cardiorespiratory afferents, alters the respiratory actions of tachykinins in the NTS.

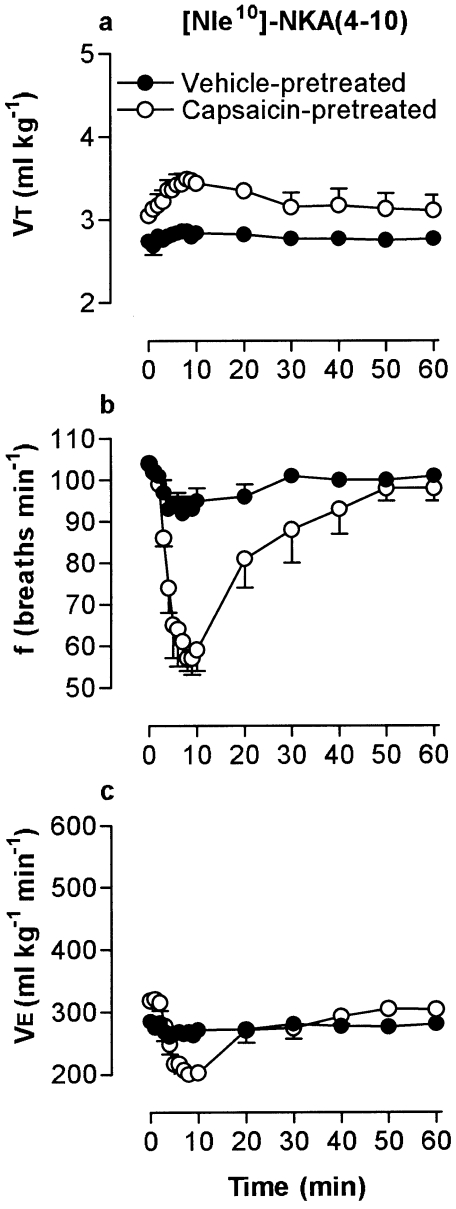

Figure 4 Respiratory response to microinjection of 300 pmol of the NK_2 receptor-selective agonist, [Nle^{10}]-NKA(4-10) into the commissural nucleus of the solitary tract of urethane-anaesthetized, spontaneously breathing adult rats (10 weeks) pretreated as neonates (day 2) with capsaicin (50 mg kg^{-1} s.c.). (a) Tidal volume (VT), (b) respiratory frequency and (c) minute ventilation (VE). Values are the mean of four animals and vertical bars show s.e.mean. Where error bars are not obvious, they are within the symbol.

Effect of capsaicin-pretreatment on the respiratory response to capsaicin

We have previously shown that microinjection of the vanilloid capsaicin into the cNTS produces severe bradypnoea, which is mediated by tachykinin NK_2 and NK_3, but not NK_1, receptors (Mazzone & Geraghty, 1999a). In the present study, this bradypnoeic response was almost abolished in adult rats which were pretreated systemically as neonates with capsaicin, but preserved in vehicle-pretreated animals.

The reduction in the sensitivity to capsaicin is indicative of a substantial loss of capsaicin-sensitive afferents in the NTS and surrounding regions. Szallasi *et al.* (1995) reported that neonatal capsaicin administration abolished [^{3}H]-RTX binding in the NTS and other brain stem regions. In addition, the respiratory response to hypoxia is markedly attenuated in adult rats pretreated as neonates with capsaicin, indicating a disruption of the peripheral chemoreceptor reflex pathway (De Sanctis *et al.*, 1991). Interestingly, we have also observed that capsaicin-induced bradypnoea is abolished in adult rats which were pretreated with capsaicin 14 days earlier, suggesting that adult-pretreatment can also disrupt afferent input to the NTS (Mazzone & Geraghty, unpublished observations). Thus, the present data provide evidence that the destruction of sensory neurons, and associated vanilloid binding sites (receptors) by neonatal capsaicin administration leads to a loss of respiratory response to central (NTS) administration of capsaicin.

Effect of capsaicin-pretreatment on the respiratory response to SP, [Sar^9,Met(O$_2$)^{11}]-SP and septide

In the present study, the VT responses to the selective NK_1 receptor agonist, [Sar^9,Met(O$_2$)^{11}]-SP, and (to a lesser extent) SP were increased in capsaicin-pretreated rats suggesting a direct or indirect input of capsaicin-sensitive afferents to NK_1 receptors in the NTS, although NK_1 receptors appear not to be involved in the direct respiratory actions of capsaicin (Mazzone & Geraghty, 1999a). In contrast, Hedner & coworkers (1985) reported that neonatal capsaicin pretreatment increased the frequency (rather than VT) response to i.c.v. administration of SP (3 μg). The reasons for these conflicting observations are unclear, but probably reflect the different routes of drug administration and initial site(s) of action of SP in the CNS.

The exaggerated responsiveness to both [Sar^9,Met(O$_2$)^{11}]-SP and SP may simply be explained by reduced enzymatic degradation (e.g., by neutral endopeptidase) in capsaicin-pretreated rats. However, this is unlikely since the peak change in VT induced by 33 pmol [Sar^9,Met(O$_2$)^{11}]-SP (2.81 ml kg^{-1}) in capsaicin-pretreated rats was greater than the maximum change induced by 10 fold higher dose in untreated rats (2.10 ml kg^{-1}; Mazzone & Geraghty, 2000). Thus, whereas 33 pmol [Sar^9,Met(O$_2$)^{11}]-SP produced a half maximal response in untreated rats, the same dose yielded a supramaximal response in capsaicin-pretreated rats. The dramatic increase in the VT response to [Sar^9,Met(O$_2$)^{11}]-SP in capsaicin-pretreated rats is more likely due to an increase in the number, or improved coupling, of postsynaptic NK_1 receptors in the NTS. Chemical or

surgical destruction of tachykinin-containing neurons, leads to an increase in the number of post-synaptic NK_1 receptors (assessed using *in vitro* autoradiography) in the rat spinal cord (Mantyh & Hunt, 1985; Yashpal *et al*., 1991; Croul *et al*., 1995). However, studies addressing the effects of surgical denervation on tachykinin receptors in the brain stem have yielded conflicting data. For example, vagotomy (which leads to destruction of some baroreceptor, chemoreceptor and pulmonary afferents) has been reported to not change or decrease the number of SP binding sites (NK_1 receptors) in the NTS (Helke *et al*., 1984; Manaker & Zucci, 1993). The present study suggests that chemical denervation (neonatal capsaicin-pretreatment) dramatically changes the responsiveness of the NTS to NK_1 receptor agonists.

In contrast to SP and [Sar^9,$Met(O_2)^{11}$]-SP, where respiratory responses were exaggerated, the VT response to another NK_1 agonist, septide, was effectively absent in capsaicin-pretreated rats. There are several reports of 'septide-sensitive' NK_1 receptors in the CNS (Steinberg *et al*., 1995; Maubach & Jones, 1997). However, to date studies have failed to determine whether the actions of septide are due to an interaction with a site different from that of SP on the classical NK_1 receptor, a NK_1 receptor conformer, or with a distinct receptor protein.

If septide-sensitive and -insensitive receptors are present in the NTS, then the present data may indicate that the capsaicin-pretreatment changes the type or conformation of the NK_1 receptor in the NTS in favour of the 'septide-insensitive' form (Maggi & Schwartz, 1997). Although the mechanisms underlying this alteration are not known, capsaicin-pretreatment may change the expression of postsynaptic NK_1 receptors in the NTS, such that there are fewer septide-sensitive binding sites present or exposed to the agonist. Alternatively, septide-sensitive NK_1 receptors may be located presynaptically in the NTS. In support of this proposal, capsaicin-pretreatment abolished only the respiratory response to septide and capsaicin. Since capsaicin is presumed to act presynaptically, then the loss of response to both agents suggests a similar synaptic locus of action. Indeed, there is both functional and immunocytochemical evidence for presynaptic tachykinin receptors which regulate the release of tachykinins and other neurotransmitters in the rat NTS and striatum and from sympathetic preganglionic neurons (Tremblay *et al*., 1992; Cammack & Logan, 1996; Jakob & Goldman-Rakic, 1996; Bailey & Jones, 1999).

Different synaptic locations of septide-sensitive and insensitive receptors may explain why capsaicin-pretreatment exaggerated the VT response to [Sar^9,$Met(O_2)^{11}$]-SP more than to SP. In vehicle-pretreated rats, the VT response to [Sar^9,$Met(O_2)^{11}$]-SP is probably mediated solely by septide-insensitive (postsynaptic) receptors, whereas the response to SP may be due to activation of both septide-sensitive (presynaptic) and -insensitive NK_1 receptors, although [Sar^9,$Met(O_2)^{11}$]-SP and SP yield similar maxima. In support of this, point mutation of the NK_1 receptor increases the affinity of septide (and NKA), without altering that of SP (Ciucci *et al*., 1998). Thus, deafferentation of the NTS by capsaicin pretreatment may upregulate septide-insensitive (postsynaptic) NK_1 receptors, resulting in the enhanced responsiveness to [Sar^9,$Met(O_2)^{11}$]-SP. In contrast, the response to SP would be reduced compared with that of [Sar^9,$Met(O_2)^{11}$]-SP since, although capsaicin-pretreatment appears to upregulate septide-insensitive receptors, there appears to be a concomitant decline in septide-sensitive NK_1 receptors.

Effect of capsaicin-pretreatment on the respiratory response to NKA and [Nle^{10}]-NKA(4-10)

Microinjection of NKA into the NTS stimulates VT, a response which appears to be mediated by NK_1 receptors since the actions of NKA are attenuated by the NK_1 antagonist RP 67580 (Mazzone & Geraghty, 2000). Furthermore, there is some evidence that NKA may act at septide-preferring NK_1 receptors, since point mutation of the NK_1 receptor increases binding affinity for both septide and NKA (Ciucci *et al*., 1998). However, respiratory responses elicited by NKA in capsaicin-pretreated rats were similar to that of vehicle-pretreated controls. The reason for this lack of change in responsiveness is unclear, since the respiratory response to septide is abolished in capsaicin-pretreated animals. One possibility is that whilst NKA may have higher affinity for septide-sensitive receptors, NKA may still interact with septide-insensitive receptors. Thus, although there appears to be a loss of septide-sensitive receptors in capsaicin-pretreated rats, a reduction in the VT response to NKA would be offset by the apparent sensitization (up-regulation) of septide-insensitive NK_1 receptors.

The respiratory response to [Nle^{10}]-NKA(4-10) is rather unique compared with all other tachykinins investigated. This selective NK_2 receptor agonist induces a bradypnoea in untreated rats, without affecting VT, which is blocked by the nonpeptide antagonist SR 48968 (Mazzone & Geraghty, 2000). In capsaicin-pretreated rats, the bradypnoeic response to [Nle^{10}]-NKA(4-10) was markedly enhanced, suggesting sensitization or upregulation of NK_2 receptors in the NTS. Interestingly, microinjection of [Nle^{10}]-NKA(4-10) increased VT in capsaicin-pretreated rats, resulting in only minor reductions in VE. The mechanisms underlying the VT response are unclear, since injection of 1 nmol [Nle^{10}]-NKA(4-10) into untreated rats produces a similar degree of respiratory slowing (approximately, -40 breaths min^{-1}) without any effect on VT (Mazzone & Geraghty, 2000). The increase in VT may simply reflect a mechanism to compensate for the marked reduction in VE which would result from the severe bradypnoea. Alternatively, the VT response may be due to an interaction between [Nle^{10}]-NKA(4-10) and NK_1 and/or NK_3 receptors, which appear to be sensitized, or increased in number, by capsaicin-pretreatment.

To date localization studies have failed to detect NK_2 receptors in the rat brain stem, although specific NK_2 immunoreactivity has recently been observed in the spinal cord where previous studies had failed to detect the receptor (Zerari *et al*., 1998). We are currently attempting to localize and quantitate NK_2 receptors in the NTS (particularly in capsaicin-pretreated rats) using immunocytochemistry and autoradiography. Indeed, if successful, up-regulating NK_2 receptors by neonatal capsaicin-pretreatment may be a novel method of studying the pharmacology of central NK_2 receptors.

Effect of capsaicin-pretreatment on the respiratory response to NKB and senktide

In the present study, capsaicin pretreatment dramatically enhanced both the frequency and VT response to NK_3 receptor agonists. Moreover, the peak stimulation of VT by senktide (3.96 ml kg^{-1}) was significantly greater than the previously observed maximum in untreated animals (2.20 ml kg^{-1}; Mazzone & Geraghty, 2000). Thus, NK_3 receptor-mediated respiratory responses also appear to be supersensitized by capsaicin-pretreatment. Again, receptor up-regulation may

explain the enhanced responsiveness to NK_3 agonists. Indeed, unilateral surgical deafferentation of L1-S2 spinal cord roots results in reactive up-regulation of dorsal horn NK_3 receptors within 1 week of the surgery (Yashpal *et al*., 1991; Croul *et al*., 1995). However, the subsequent sensitivity of spinal cord neurons to NK_3 agonists was not determined.

Although NK_3 receptors are undoubtedly involved in the respiratory response to microinjection of NKB and senktide into the cNTS, the role of NK_3 receptors in reflex integration in the NTS is unclear. Moreover, there is some uncertainty as to the source of endogenous NKB which activates NK_3 receptors, since NKB is not contained in primary afferent neurons in the NTS (Nagashima *et al*., 1989). NKB may be involved in secondary integration or contained in neurons which project to the NTS and play a role in modifying primary transmission. Alternatively, although the actions of SP and NKA in untreated rats are not altered by the NK_3 receptor antagonist SR 142801 (Mazzone & Geraghty, 2000), endogenous tachykinins are notoriously promiscuous and may activate NK_3 receptors at physiological concentrations (see Mussap *et al*., 1993). In either case, NK_3 receptors do not appear to be synaptically located in the rat NTS (Carpentier & Baude, 1996), suggesting a complex role for NK_3 receptors in the reflex control of respiration.

In conclusion, these studies suggest that neonatal capsaicin pretreatment sensitizes the NTS to selected NK_1, NK_2 and NK_3 tachykinin receptor agonists. The mechanisms underlying this sensitization are not known, but may represent reactive receptor up-regulation following chemical deafferentation of the NTS. Moreover, the apparent loss of septide responsiveness in capsaicin-pretreated rats suggests that a population of septide-sensitive NK_1 receptors are located presynaptically on capsaicin-sensitive respiratory afferents in the NTS.

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