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Functional $GABA_A$ receptors on rat vagal afferent neurones

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1 In the present study, *in vitro* electrophysiology and receptor autoradiography were used to determine whether rat vagal afferent neurones possess γ-aminobutyric acid $(GABA)_A$ receptors.

2 GABA (1–100 μM) and isoguvacine (3–100 μM) caused a concentration-dependent depolarization of the rat isolated nodose ganglion preparation at room temperature. When applied to the tissue 20 min before the agonist, SR95531 (3 μM) and bicuculline (3 μM) caused a parallel shift to the right of the GABA and isoguvacine concentration-response curves, yielding shifts of 81 fold and 117 fold for SR95531 and 4 fold and 12 fold for bicuculline, respectively.

3 Baclofen (10 nM–100 μM) was unable to elicit a depolarization of the rat isolated nodose ganglion preparation at either room temperature or at 36°C, whilst 5-aminovaleric acid (10 μM), a $GABA_B$ receptor antagonist, was unable to antagonize significantly the GABA-induced depolarization at either room temperature or at 36°C.

4 [^{3}H]-SR95531 (7.2 nM), a $GABA_A$ receptor-selective antagonist, bound topographically to sections of rat brainstem. Specific binding was highest in the medial nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus nerve (DMVN). Binding was also observed in certain medullary reticular nuclei, in particular the parvocellular reticular nucleus.

5 Unilateral nodose ganglionectomy caused a reduction in $GABA_A$ binding site density in the medial NTS from 93 ± 7 to 68 ± 6 d.p.m./mm^2. This procedure also caused a reduction in $GABA_A$ binding site density in the side of the NTS contralateral to the lesion, from 151 ± 12 to 93 ± 7 d.p.m./mm^2. Sham surgery had no effect on the binding of [^{3}H]-SR95531 in rat brainstem.

6 The present data provide evidence for the presence of $GABA_A$ receptors located on the soma and central terminals of rat vagal afferent neurones. Additionally, a population of $GABA_A$ receptors is evidenced postsynaptically in the rat NTS with respect to vagal afferent terminals. These data are discussed in relation to the functional pharmacology of GABA in this region of the NTS.

Keywords: γ-Aminobutyric acid (GABA); $GABA_A$ receptors; [^{3}H]-SR95531; nodose ganglion; vagus nerve; nucleus tractus solitarius; *in vitro* electrophysiology; autoradiography

Introduction

Within the central nervous system, γ-aminobutyric acid (GABA) is the most important inhibitory amino acid neurotransmitter, being used in between 20 and 50% of central synapses depending upon brain region (Bloom & Iversen, 1971). Not surprisingly therefore, GABA is implicated in a multiplicity of effects, including cardiovascular homeostasis (Sved, 1994). In respect of central cardiovascular function, the nucleus tractus solitarius (NTS), located in the medulla oblongata and serving as an integrative relay for the baroreceptor heart-rate reflex (Spyer, 1990) has been found to contain relatively high levels of GABA compared to other brain regions (Siemers *et al*., 1982). In addition, numerous studies have indicated the importance of GABA, acting via both $GABA_A$ and $GABA_B$ receptors in this nucleus in both tonic maintenance of blood pressure (Bousquet *et al*., 1982; Catelli *et al*., 1987; Merahi *et al*., 1992) and baroreceptor heart-rate reflex functioning (Florentino *et al*., 1990; Sved & Tsukamoto, 1992).

In respect of the peripheral control of the cardiovascular function, intravenous GABA has been observed to reduce vagally mediated bradycardia in the anaesthetized rat (Maggi *et al*., 1985), systemic picrotoxin or bicuculline to cause a pressor effect in spinal rats (Wible & DiMicco, 1986) and systemic GABA to decrease blood pressure and heart rate in anaesthetized dogs (Vemulapalli & Barletta, 1984). The afferent component of the baroreceptor heart-rate reflex is mediated by the transmission of nervous impulses generated by barosensitive neurones located in the aortic arch and carotid bifurcation along the vagus and glossopharyngeal nerves respectively (Lawrence & Jarrott, 1996). Neuronal cell bodies of centrally projecting vagal afferents, including baroafferents, are located in the nodose ganglion (Spyer, 1990; Lawrence & Jarrott, 1996). The presence of GABA has been detected in the nodose ganglion both chemically (Bertilsson *et al*., 1976) and immunocytochemically (Szabat *et al*., 1992). Functionally, GABA has been shown to depolarize rabbit nodose ganglion cells (Wallis *et al*., 1982), an effect which was found to be picrotoxin sensitive. The possibility that this effect is mediated by the action of GABA on $GABA_A$ receptors was not investigated further. The aims of this present study were to investigate more fully the vagal pharmacology of GABA by the use of both functional and anatomical techniques in the rat. Functionally, the isolated nodose ganglion/vagus grease-gap technique was employed to determine whether activation of $GABA_A$ receptors on the soma membrane by both the endogenous ligand and the $GABA_A$ receptor-selective agonist isoguvacine (Kemp *et al*., 1986) could evoke an electrophysiological response. Since this methodology can also indicate the presence and properties of receptors on central vagal afferent terminals (Round & Wallis, 1986), the $GABA_A$ receptor-selective antagonist [^{3}H]-SR95531 (Heaulme *et al*., 1987) was used in autoradiographic experiments to determine the distribution of $GABA_A$ binding sites in the rat brain stem. Unilateral vagal deafferentation was performed to determine whether labelled sites were pre- or post-synaptic in location with respect to vagal afferent terminals.

Methods

All of the experiments described here were performed in accordance with the Prevention of Cruelty to Animals Act 1986

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under the guidelines of the NH & MRC Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia.

In vitro *electrophysiology*

Electrophysiological experiments were performed as previously described (Castillo-Meléndez *et al*., 1994; Lawrence *et al*., 1995). In brief, male Wistar-Kyoto rats (250–350 g) were killed by cervical dislocation and the nodose ganglia with attached vagal trunk were removed and desheathed. The tissue was then placed in a twin chambered perspex bath with the nodose ganglion placed in one compartment of the bath and isolated from the vagal nerve trunk in an adjacent compartment by a silicone grease seal, as previously described (Castillo-Melendez *et al*., 1994). The preparation was superfused with Krebs buffer (room temperature or 36°C, as stated in relevant results sections, 2 ml min^{-1}) of the following composition (mM): NaCl 118, $NaHCO_3$ 24.9, KH_2PO_4 1.3, KCl 4.7, $CaCl_2$ 2.6, glucose 11, $MgSO_4$ 1.2, gassed with 95% O_2/5% CO_2, pH approximately 7.4. The d.c. potential between the two compartments following drug administration to the nodose ganglion was recorded by calomel electrodes connected to the preparation through agar-KCl bridges. The potential changes were amplified and displayed on a Grass Polygraph (Model 79D). Drugs were applied non-cumulatively and remained in contact with the tissue until apparent equilibrium was reached. This was followed by a washout and recovery period (10–20 min) to allow full repolarization before another drug addition. When used, antagonists were added to the superfusate 20 min before agonist application. This method allowed stable responses to be measured over a 5–6 h period. The magnitude of an observed depolarization was measured from a projection of the baseline preceding the response to account for any drift in the preparation. At the beginning and end of each experiment a positive control of a previously determined maximal concentration of 5-HT (3 μM; Castillo-Melendez *et al*., 1994) was applied to the nodose ganglia in order to check the viability of the preparations.

Unilateral vagal deafferentation

Male Wistar-Kyoto rats (250–350 g) were anaesthetized with sodium methohexitone (60 mg kg^{-1}, i.p.) and placed on their back. A midline incision was made in the neck and left nodose ganglion was exposed and excised ($n=5$) including trunks of vagus, superior laryngeal and inferior pharyngeal nerves at the respective points of contact with the nodose ganglion. In a second group ($n=5$), the left nodose ganglion was exposed but not removed (sham control). After a 14 day recovery period, the animals were re-anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), immediately killed by decapitation and their brainstems removed. All tissue was frozen over liquid nitrogen at the time of removal and stored at −80°C until further processed.

In vitro *autoradiography*

Cryostat-cut sections of brainstem (10 μm) were taken between bregma −14.4 mm and bregma −13.4 mm. Autoradiography experiments were carried out by use of a modified version of a previous protocol (Bristow & Martin, 1988). Briefly, tissue sections were allowed to equilibrate to room temperature and pre-incubated (30 min, room temperature) in Tris-citrate buffer (50 mM, pH 7.4) to remove endogenous GABA. The sections were then cooled to 4°C and incubated with [^{3}H]-SR95531 (7.2 nM) for 55 min in buffer of the same composition as that used for the pre-incubation. Non-specific binding was determined as that remaining in the presence of GABA (10 mM). The slide-mounted sections were then washed in ice-cold Tris-citrate buffer (50 mM, pH 7.4, 3 × 5 s) followed by rinsing in ice-cold distilled water (2 × 10 s). Following this, the sections were dried under a gentle stream of cool air and dessicated overnight. Subsequent to drying, slides were apposed to tritium sensitive film (Amersham Hyperfilm) in the presence of tritium microscales (Amersham) for 4 weeks.

Developed autoradiograms were quantified by an MCID M4 image analysis system (Imaging Research Inc., Canada), by comparison of the optical densities of autoradiographic images with those of the standard microscales. Photographs of autoradiograms were scanned into a personal computer, imported into Adobe Photoshop, enhanced and printed. Brain regions were identified following microscopic examination of adjacent tissue sections stained with 0.1% thionin with reference to a stereotaxic atlas (Paxinos & Watson, 1986).

Materials

5-Aminovaleric acid, bicuculline, baclofen, isoguvacine and SR95531 (2-(3′-carboxyl-2′-propyl)-3-amino-6-*P*-methoxyphenylpyridazinium bromide) were obtained from Research Biochemicals Inc. (Natick, MA, U.S.A.), GABA from Sigma Chemicals, (St. Louis, MO, U.S.A.) and [^{3}H]-SR95531 from Dupont NEN (Wilmington, DE, U.S.A.). Other reagents were of either laboratory or analytical grade from various suppliers.

Statistics

Concentration-response curves on the isolated nodose ganglion preparation were analysed by potency ratios. A paired t test (two-tailed) was employed to analyse differences in [^{3}H]-SR95531 binding between the denervated and intact sides of the NTS. An unpaired t test (two-tailed) was employed to analyse differences in [^{3}H]-SR95531 binding between intact

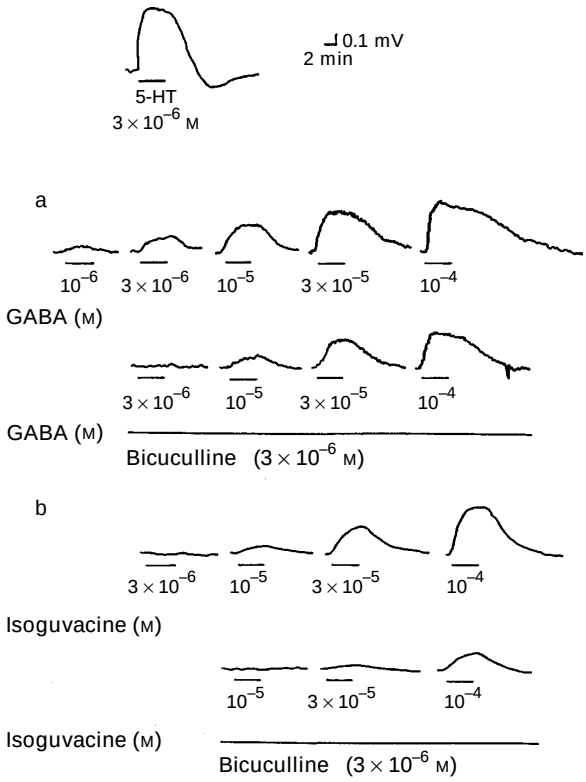

Figure 1 Polygraph trace showing (a) a representative series of responses to different concentrations of GABA (1–100 μM) applied to the rat isolated nodose ganglion preparation and the effect of addition of bicuculline (3 μM) to the preparation on this series of responses. (b) A representative series of responses to isoguvacine (3–100 μM) applied to the rat isolated nodose ganglion preparation and the effect of addition of bicuculline (3 μM) to the preparation on this series of responses. The first depolarization represents an initial positive control response to 5-hydroxytryptamine (5-HT, 3 μM). Scales as shown.

sides of the NTS in sham and nodose ganglionectomized rats. In all cases, $P<0.05$ was considered significant.

Results

In vitro *electrophysiology*

Addition of either GABA, or the $GABA_A$ receptor agonist isoguvacine to the superfusate bathing the isolated nodose ganglia elicited a concentration-dependent depolarisation at room temperature (Figure 1). Addition of the $GABA_B$ receptor agonist baclofen (10 nM – 100 μM) to the superfusate elicited no response, either at room temperature or at 36°C (results not shown), thus discounting the possibility of adenylate cyclase inactivity at room temperature. Over the concentration range employed in the study (1 – 100 μM GABA, 3 – 100 μM isoguvacine) neither compound consistently achieved an apparent maximum response. Data were therefore treated in the same manner as previously described (Lawrence *et al*., 1995); i.e. data were quantified in terms of potency ratios (utilising the programme COMPAR) by comparing the complete concentration-response curves obtained for agonists in the absence and presence of antagonists. The $GABA_A$ receptor selective antagonists SR95531 (3 μM) and bicuculline (3 μM) shifted the concentration response curves for both GABA and isoguvacine to the right in a parallel fashion. For example, at a concentration of 30 μM GABA, inclusion of SR95531 ($n=7$) or bicuculline ($n=5$) in the superfusate resulted in a reduction in the depolarization from 0.54 ± 0.07 mV to 0.07 ± 0.03 mV and 0.65 ± 0.10 mV to 0.29 ± 0.04 mV, respectively. Analysis of the complete concentration-response curves yielded potency ratios of 0.012 and 0.252, respectively, for SR95531 and bicuculline against GABA. These rightward shifts in the efficacy of GABA translated into an overall 81 fold rightward shift in the presence of SR95531 and an overall 4 fold rightward shift in the presence of bicuculline (Figure 2). In contrast to the effects of SR95531 and bicuculline, the $GABA_B$ receptor-selective antagonist 5-aminovaleric acid (10 μM) had no effect upon GABA induced depolarisations, either at room temperature ($n=4$) or at 36°C ($n=4$), again discounting the possibility of adenylate cyclase inactivity at room temperature (Figure 2).

Similarly, the two $GABA_A$ receptor antagonists attenuated the ability of isoguvacine to evoke an electrophysiological response of the rat isolated nodose ganglion preparation. Thus, at a concentration of 100 μM isoguvacine, inclusion of SR95331 ($n=6$) or bicuculline ($n=6$) in the superfusate resulted in a reduction in the depolarisation from 0.40 ± 0.06 mV to 0.14 ± 0.02 mV and 0.69 ± 0.09 mV to 0.27 ± 0.05 mV, yielding potency ratios of 0.008 and 0.083, respectively, for SR95531 and bicuculline against isoguvacine. These rightward shifts in the efficacy of isoguvacine translated into an overall 117 fold rightward shift in the presence of SR95531 and an overall 12 fold rightward shift in the presence of bicuculline (Figure 3).

In vitro *autoradiography*

The binding profile of [^{3}H]-SR95531 has previously been characterized (Heaulme *et al*., 1987) and in our study, it bound specifically to sections of rat brainstem. GABA (10 mM) markedly inhibited the binding of [^{3}H]-SR95531 such that non-specific binding was not able to be quantified on the MCID M4 image analysis system (Figure 4d). Specific binding of [^{3}H]-SR95531 was predominantly localized to the NTS, DMVN and certain medullary reticular nuclei, in particular the parvocellular reticular nucleus. At the most caudal anatomical levels of the region of the brainstem analysed (bregma −14.4 mm), unilateral deafferentation of the vagus had little or no effect on the density of GABA sensitive [^{3}H]-SR95531 binding sites in the NTS when intact NTS was compared to

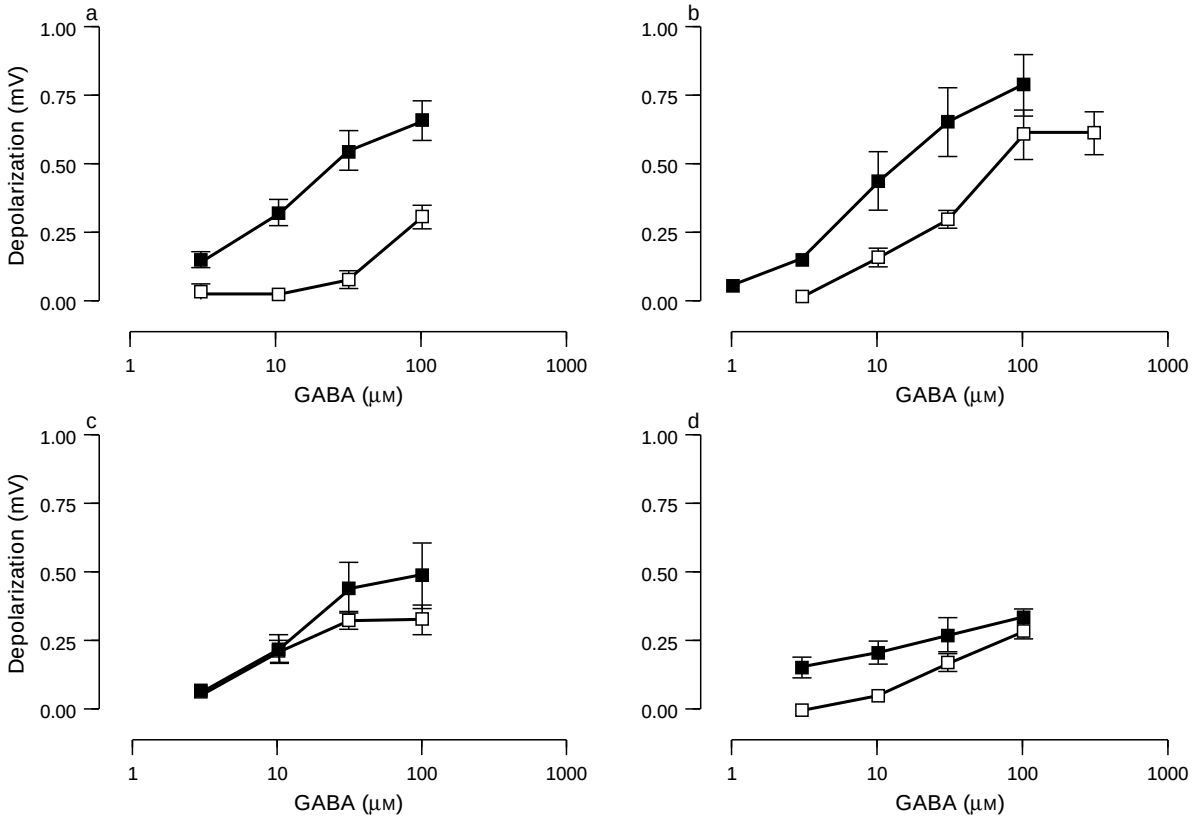

Figure 2 Concentration-response curves for GABA in the absence (■) or presence (□) of: (a) SR95531 (3 μM, $n=7$), (b) bicuculline (3 μM, $n=5$) and 5-aminovaleric acid (10 μM) at either (c) room temperature ($n=4$) or (d) 36°C ($n=4$). Data points are mean and vertical lines show s.e.mean.

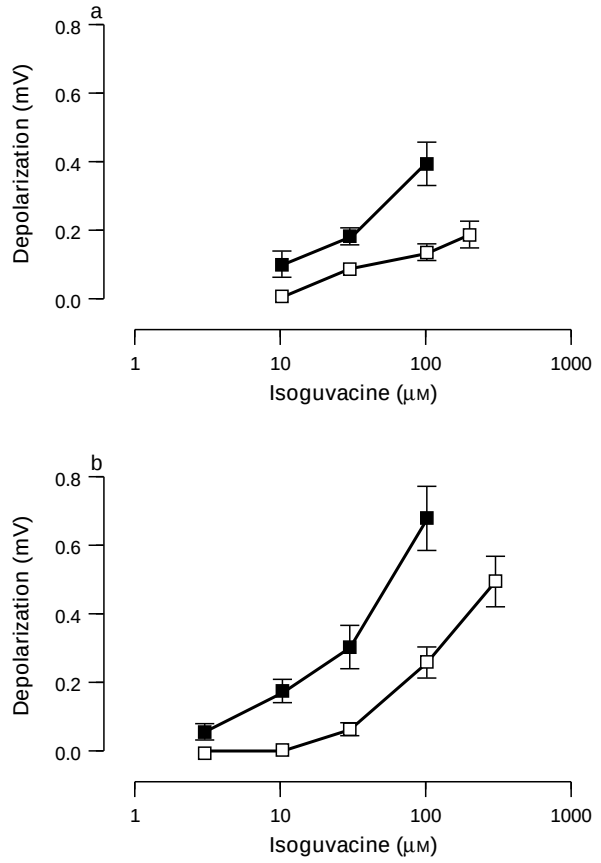

Figure 3 Concentration-response curves for isoguvacine in the absence (■) or presence (□) of: (a) SR95531 (3 μM, $n=6$) and (b) bicuculline (3 μM, $n=6$). Data points are mean and vertical lines show s.e.mean.

denervated NTS in the same animal. However, between bregma −13.8 mm and bregma −13.4 mm, unilateral nodose ganglionectomy caused a significant reduction in GABA-sensitive [^{3}H]-SR95531 binding sites. This was greatest at bregma −13.6 mm where the density of binding sites was reduced from 93 ± 7 (contralateral) to 68 ± 6 (ipsilateral) d.p.m./mm^2 ($P<0.001$ paired t test, intact versus denervated NTS, $n=3$ sections per animal from 5 animals). An example autoradiogram is shown in Figure 4b. At this level the specific binding of [^{3}H]-SR95531 was distributed throughout all subnuclei of the NTS, namely the medial, dorsal, intermediate, ventral, gelatinous, interstitial, dorsolateral, lateral and ventrolateral subnuclei. In contrast, sham surgery did not affect GABA-sensitive binding of [^{3}H]-SR95531 in the rat NTS ($n=3$ sections per animal from 5 animals, Figure 4a). However, on comparing GABA-sensitive [^{3}H]-SR95531 binding sites in the NTS on the intact side between sham operated and nodose ganglionectomized animals, a reduction was observed to occur. This reduction occurred between bregma −14.4 mm and bregma −13.8 mm, and was greatest at bregma −13.8 mm where binding was reduced from 151 ± 12 to 93 ± 7 d.p.m./mm^2 ($P<0.001$ unpaired t test, sham operated intact NTS versus nodose ganglionectomised intact NTS, $n=3$ sections per animal from 10 animals).

Discussion

The present study has provided the first direct evidence for the involvement of GABA$_A$ receptor mediated events in neurotransmission of rat vagal afferent neurones. Functionally, the presence of GABA$_A$ receptors on the nodose ganglion is in agreement with previous findings from studies on rabbit nodose ganglion (Wallis *et al*., 1982), although the criteria by which this demonstration has been made are somewhat more stringent i.e. the concentration-response curves to both GABA and the selective GABA$_A$ receptor agonist isoguvacine were shifted to the right in a parallel manner by the selective GABA$_A$ receptor antagonists SR95531 and bicuculline. The order of potency in magnitude of shift achieved by each antagonist is in agreement with their previously obtained K_D values (Heaulme *et al*., 1986). The autoradiographic experiments, in conjunction with nodose ganglionectomy, confirm the presence of populations of GABA$_A$ binding sites located both pre- and post-synaptically with respect to vagal afferent terminals within the rat NTS. Binding sites were also observed postsynaptically within the dorsal motor nucleus of the vagus nerve (DMVN), this being one of the two locations of the preganglionic parasympathetic motorneurones providing vagal outflow to the viscera (Van Giersbergen *et al*., 1992). The validity of this technique for the determination of the anatomical location of neurotransmitter receptor populations is well established; it has previously been used to demonstrate the presynaptic localization of dopamine D$_2$ receptors (Lawrence *et al*., 1995), adenosine A$_{2a}$ receptors (Castillo-Melendez *et al*., 1994), angiotensin II receptors (Lewis *et al*., 1986), vasopressin V$_1$ receptors (Gao *et al*., 1992), glutamate receptors (Lewis *et al*., 1988), neurotensin receptors (Kessler & Beaudet, 1989), cholecystokinin receptors (Ladenheim *et al*., 1988), 5-HT$_3$ receptors (Merahi *et al*., 1992) and substance P receptors (Manaker & Zucchi, 1993).

Both GABA$_A$ and GABA$_B$ binding sites have previously been demonstrated to be present throughout the brain, including, at the level of the brainstem, the spinal trigeminal nucleus, NTS and DMVN (Gale *et al*., 1980; Bowery *et al*., 1987). However, to date, the subnuclear distribution of GABA$_A$ binding sites within the NTS has not been addressed, in spite of its highly subdivided nature. The localization of GABA$_A$ binding sites in the rat brain stem subsequent to unilateral nodose ganglionectomy has been shown in one previous study (Pratt & Bowery, 1992). In this study, which made use of [^{3}H]-GABA in the presence of the GABA$_B$ receptor agonist baclofen, GABA$_A$ binding sites were found to be located both pre- and post-synaptically with respect to vagal afferent terminals. However, the greatest density of postsynaptic GABA$_A$ binding sites prevailed in the DMVN rather than in the NTS. [^{3}H]-GABA also binds to GABA receptors on glial cells, neuronal uptake sites and GABA metabolizing enzymes (Mohler & Okada, 1978) and may not therefore be the ideal ligand for determining GABA binding sites representative of functional receptor populations. This may have contributed to a difference in the observed topographical distribution of binding sites between the current study and that of Pratt & Bowery (1992). Taking the autoradiographic and functional findings of this study together, it would appear that GABA$_A$ receptors are synthesized in nodose perikarya and axoplasmically transported to the central nerve terminals in the NTS. More specifically, the binding results at each anatomical level of the NTS studied suggest that this process is confined largely to the medial NTS. In support of this hypothesis, work is presently underway in our laboratory to investigate the localisation of the mRNA encoding the various subunits of the GABA$_A$ receptor complex within the rat nodose ganglion.

Whilst the electrophysiological methodology employed does not allow the identification of neuronal function, the observed response representing a population rather than individual effect, there are reasons to suggest that at least part of the observed functional effect of GABA is mediated via an action on GABA$_A$ receptors located on baroreceptive neurones. Firstly, it has been previously observed that systemic administration of either bicuculline or picrotoxin in the rat results in a vagally mediated hypertension (Wible & DiMicco, 1986). Secondly, the observed reduction in density of GABA$_A$ binding sites in the present study occurs within the medial region of the NTS, a subregion that is involved in baroreceptor heart rate-reflex pathway function (Van Giersbergen *et al*., 1992; Lawrence &

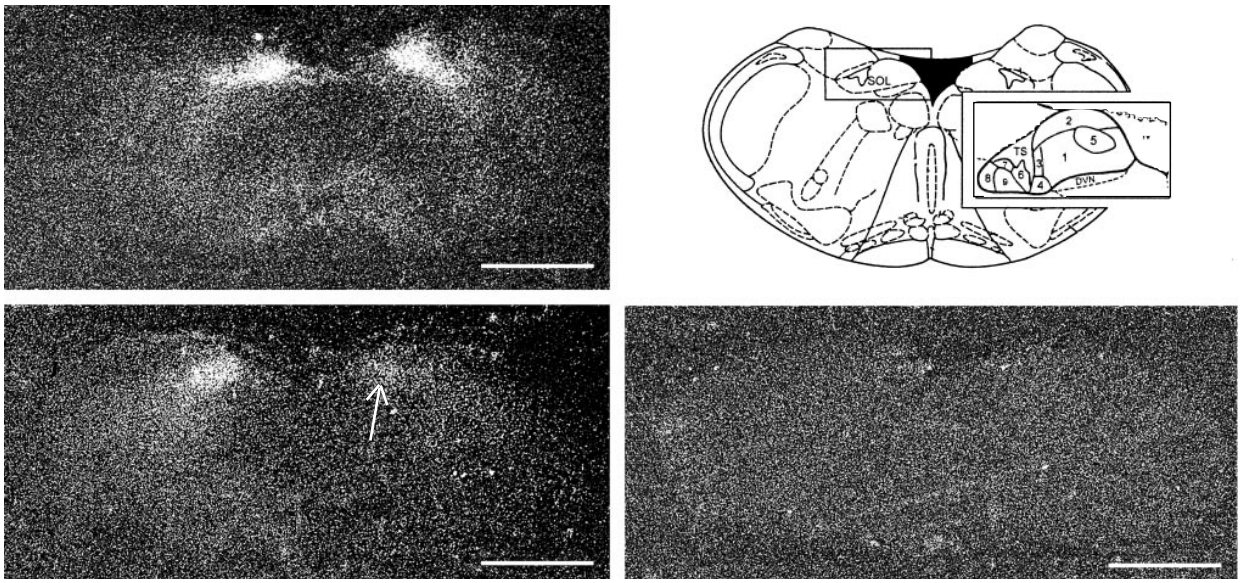

Figure 4 Computer enhanced autoradiograms of [^{3}H]-SR95531 binding to sections of rat brainstem. (a) Sham nodose ganglionectomy control. Anatomical level corresponds to bregma −13.4 mm. $GABA_A$ binding sites are predominantly located in the dorsal vagal complex (NTS and DMVN). Scale bar = 1.4 mm. (b) Unilateral nodose ganglionectomy. Anatomical level corresponds to bregma −13.4 mm. A reduction in $GABA_A$ binding site density can be observed in the denervated side of the dorsal vagal complex (arrowhead) compared to the contralateral side. Scale bar = 1.4 mm. (c) Schematic drawing showing subdivisions of the rat NTS at the anatomical level of the autoradiograms. A complete section of brainstem shows the relative position of the NTS on the dorsal surface of the brainstem. The boxed area is shown inset to allow definition of NTS subnuclei. Annotation as follows: (1) medial; (2) dorsal; (3) intermediate; (4) ventral; (5) gelatinous; (6) interstitial; (7) dorsolateral; (8) lateral; (9) ventrolateral; DVN, dorsal vagal nucleus; TS, solitary tract. Adapted from Paxinos and Watson (1986) and Van Giersbergen *et al.* (1992). (d) Non-specific binding in the presence of GABA (10 mM).

Jarrott, 1996). Thirdly, local administration into the NTS of either $GABA_A$ receptor selective agonists or antagonists has been shown to cause hypertension or hypotension, respectively (Bousquet *et al*., 1982; Catelli *et al*., 1987).

However, the location of $GABA_A$ receptors mediating these effects within the NTS has not been fully addressed; the site of location of these receptors may exist either pre- or post-synaptically in respect of primary baroreceptor afferents. Presently, functional evidence obtained from studies in which effects of GABA or its agonists and antagonists on blood pressure and heart rate are observed to be mediated through $GABA_A$ receptors (Bousquet *et al*., 1982; Catelli *et al*., 1987) does not allow one to ascertain whether the relevant receptors are located pre- or post-synaptically. However, electrophysiologically, bicuculline has been demonstrated to increase the activity of NTS neurones (Bennett *et al*., 1987; McWilliam & Shpeheard, 1988) and to potentiate evoked responses in barosensitive neurones in the NTS (Suzuki *et al*., 1993), thus implicating mediation through postsynaptically located $GABA_A$ receptors. These postsynaptically located $GABA_A$ receptors may be present on either GABAergic interneurones, which themselves receive a GABAergic input (Maqbool *et al*., 1991), or on neurones projecting from the NTS to other brain nuclei involved in central cardiovascular function; the caudal ventrolateral medulla for example (Van Giersbergen *et al*., 1992). However, the importance of presynaptically located $GABA_A$ receptors, whilst lacking in functional evidence, cannot be discounted, especially given the fact that a proportion of the total observed binding sites (approximately 30%) are located presynaptically on vagal afferent terminals.

The effects of GABA on cardiovascular function within the NTS, as previously stated, are not exclusively mediated through an action on $GABA_A$ receptors. A similar body of evidence implicates a role for $GABA_B$ receptors in this brain region. Thus, local administration into the NTS of the $GABA_B$ receptor agonist baclofen causes a pressor effect that is reversed by phaclofen or CGP35348 (Florentino *et al*., 1990; Trippenbach & Lake, 1994), or an inhibiton of baroreceptor reflexes (Lalley, 1980). Indeed, the pressor effect caused by application of the GABA uptake blocker, nipecotic acid is mediated by bicuculline-insensitive GABA receptors (Sved & Sved, 1990) suggesting that under conditions of increased levels of endogenous GABA, the $GABA_B$ receptor is of greater importance than the $GABA_A$ receptor. It is possible that, physiologically, the $GABA_A$ receptor is of more importance in actions such as the reported inhibition of barosensitive neurones elicited by stimulation of the hypothalamic defence area (Jordan *et al*., 1988), an effect of which can be blocked by the application into the NTS of either bicuculline of 2-OH-salcofen (Kunos & Varga, 1995). Regarding the location of relevant $GABA_B$ receptors, vagal deafferentation in rats has been observed to result in a decrease in $GABA_B$ binding site density in the NTS, suggesting the presence of both a pre- and post-synaptic population of sites (Bowery & Pratt, 1992).

Whilst evidence for a presynaptic modulation of aortic baroreceptor afferents is lacking, as assessed by threshold to antidromic activation (Richter *et al*., 1986), it has been found that $GABA_B$ receptors are involved in both pre- and post-synaptic actions in the NTS, as baclofen was observed to reduce both evoked and spontaneous synaptic transmission in rat brainstem slices at concentrations where no direct post-synaptic depolarization was discernible (Brooks *et al*., 1992). However, our results indicate that on the nodose ganglion itself, $GABA_A$ receptors are functionally more relevant than $GABA_B$ receptors; baclofen was unable to evoke an electrophysiological response over the concentration range studied whilst 5-aminovaleric acid was unable to antagonize the depolarization caused by GABA.

The favoured neutransmitter at primary baroreceptor afferent terminals of the vagus nerve is L-glutamate (Talman *et al*., 1980; Lawrence & Jarrott, 1994). Evidence from immunocytochemical studies in the cat indicates that GABAergic terminals form synapses on the soma or dendrites of neurones within the NTS, and that either glutamate immunoreactive terminals (potential baroafferent terminals) or horseradish peroxidase-labelled vagal afferents synapsed on the same soma or dendrites (Maqbool *et al*., 1991; Saha *et al*., 1995). Whilst these results provide an anatomical basis for GABA mediated

modulation of glutamatergic or vagal input to the NTS, little evidence of axo-axonic synapse formation between GABA and glutamatergic immunoreactive neurones, indicative of presynaptic inhibition, was found.

Thus, present anatomical evidence is not supportive of a role in central cardiovascular function of the presynaptic population of binding sites evidenced in this study. Whilst this is at odds with the observation that the reduction in $GABA_A$ binding sites following vagal deafferentation predominates in the medial NTS, the subregion of the NTS involved predominantly in cardiovascular regulation, the presence of $GABA_A$ binding sites on vagal afferent terminals involved in respiratory, gustatory or gastrointestinal homeostasis is a possibility. In particular, with respect to the region of the NTS studied, the involvement of a presynaptic modulation of neurones in respiratory control must be considered. Functional evidence indicates that respiratory vagal afferent fibres are subject to presynaptic influence (Richter *et al*., 1986), whilst immunocytochemical evidence demonstrates a relationship between GABAergic fibres and inspiratory neurones of the dorsal respiratory group (Lipski *et al*., 1990).

The autoradiographic results also support the notion that the NTS receives a partial bilateral innervation by the left vagus as $GABA_A$ binding sites in the NTS were significantly reduced in the ganglionectomised animals both ipsilateral and contralateral to the lesion. This finding is in agreement with other work from our laboratory in which a dextran biotin tracer injected into the left nodose ganglion was visualised in both the left and right medial NTS (unpublished observation). The physiological relevance of this finding is not known but may relate to cross-talk between the right and left vagus at the level of the NTS.

In conclusion, the present study has determined the ability of GABA to depolarise rat vagal afferent neurones in a concentration-dependent manner. The action of GABA appears to be mediated by activation of $GABA_A$ receptors. Additionally, the binding of [^{3}H]-SR95531 to rat brain stem has provided anatomical confirmation of the presence of presynaptic $GABA_A$ binding sites on central afferent terminals of the vagus nerve.

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