Differential regulation of $GABA_A$ and $GABA_B$ receptors during vestibular compensation

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We investigated changes in intrinsic excitability and GABA receptor efficacy in rat medial vestibular nucleus (MVN) neurons following 48 h and 7–10 days of behavioral recovery after unilateral labyrinthectomy (UL) in the rat. The mean *in vitro* discharge rate of rostral ipsilesional MVN cells at both time points was significantly higher than normal, indicating that the intrinsic excitability of the deafferented cells undergoes a sustained up-regulation during vestibular compensation. In slices from animals that had compensated for 7–10 days after UL, the responsiveness of rostral ipsilesional MVN cells to the $GABA_A$ agonist muscimol was not different from normal, while the responsiveness to the $GABA_B$ agonist baclofen was significantly down-regulated. This is in contrast to the situation soon after UL, where the efficacy of both $GABA_A$ and $GABA_B$ receptors is markedly down-regulated. The recovery of fast $GABA_A$ mediated neurotransmission by 7–10 days post-UL presumably enables ipsilesional cells to again respond to vestibular stimulation, through commissural inhibitory modulation from the intact side. The permanent loss of excitatory input from the lesioned side may be, in effect, counteracted by the long-term down-regulation of slow $GABA_B$ receptors in the de-afferented neurons. *NeuroReport* 12:597–600 © 2001 Lippincott Williams & Wilkins.

Key words: GABA receptors; Medial vestibular nucleus; Plasticity; Unilateral labyrinthectomy; Vestibular compensation

INTRODUCTION

Damage to one vestibular nerve or the vestibular receptors of one inner ear results in severe oculomotor and postural symptoms including spontaneous ocular nystagmus, circular walking, roll and yaw head-tilt, and falling toward the lesioned side. Many of these immediate consequences of unilateral labyrinthectomy (UL) subside rapidly and disappear over 2–3 days, through a process of CNS plasticity known as vestibular compensation (VC). The cellular mechanisms responsible for VC have been the subject of much interest [1–3]. Recent studies have shown that significant adaptive changes occur in the properties of brain stem medial vestibular nucleus (MVN) neurons in the early stages of VC, that are likely to be instrumental in restoring their resting activity to near normal levels after UL [4–6]. Using slices of the rat MVN prepared from animals at various times after UL, these studies provided direct experimental evidence for two distinct but synergistic mechanisms of plasticity. Within 4 h post-UL, rostral ipsilesional MVN neurons developed a significant increase in their intrinsic excitability, which was observed in slices as an elevated spontaneous *in vitro* resting discharge rate [4,5]. Intracellular experiments showed this increased intrinsic excitability to be due to changes in the resting membrane potential and input resistance of the ipsilesional MVN cells as well as changes in their active membrane conductances after UL [7]. Also within 4 h post-UL, rostral ipsilesional MVN neurons showed a marked down-regulation of their responsiveness to the $GABA_A$ and $GABA_B$ receptor agonists muscimol and baclofen [6]. The rostral region of the MVN is richly innervated by primary horizontal semicircular canal afferents, and contains predominantly neurons concerned with the vestibulo-ocular reflex (VOR) including flocculus target neurons, and neurons involved in the reciprocal commissural inhibitory system [8]. We have proposed that the increased intrinsic excitability of ipsilesional MVN neurons, together with the decrease in inhibitory receptor efficacy, may synergistically counteract the disfacilitation and excessive commissural inhibition to which these neurons are subjected immediately after the lesion [4,6].

In this study we extended our investigation of the changes in intrinsic excitability and the functional efficacy of GABA receptors in MVN neurons to the longer term (48 h and 7–10 days) post-UL, to determine if the rapid adaptive changes that occur within 4 h post-UL persist after the initial compensation of the immediate symptoms is complete. We compared the responsiveness of ipsilesional and contralesional MVN neurons to muscimol and baclofen, in slices prepared from animals that had compen-

sated for 7–10 days after UL. The results show that, in contrast to the position in the short term after UVD where the functional efficacy of both $GABA_A$ and $GABA_B$ receptors is markedly down-regulated [6], after 7–10 days post-UL the responsiveness of the ipsilesional MVN cells to muscimol has returned to normal while the responsiveness to baclofen remains down-regulated. We suggest that the recovery of fast $GABA_A$ mediated inhibitory neurotransmission presumably enables the ipsilesional cells to again respond to vestibular stimulation, through commissural inhibitory modulation from the intact side; while the permanent loss of excitatory input from the lesioned primary vestibular afferents may be, in effect, partly compensated for by a sustained down-regulation of the efficacy of slow $GABA_B$ inhibitory receptors in the deafferented neurons.

MATERIALS AND METHODS

Extracellular recordings of the spontaneous tonic activity of MVN neurones were made in horizontal slices of the rat dorsal brain stem *in vitro*, using techniques similar to those described earlier [4–6]. Slices of the MVN were prepared from Sprague–Dawley rats (100–160 g) that had undergone a left unilateral labyrinthectomy under avertin (tribromoethanol, 300 mg kg^{-1}) anaesthesia either 48 h or 7–10 days earlier. The labyrinthectomy was carried out by opening the horizontal semicircular canal duct in the temporal bone with a 0.7 mm diameter dental drill, following the open duct anteriorly to the vestibule of the inner ear, and aspirating the contents of the vestibule which was then rinsed with 100% ethanol. Recovery from avertin anaesthesia was complete 30–40 min after induction. Labyrinthectomized animals showed the characteristic behavioural symptoms of unilateral vestibular loss, including spontaneous ocular nystagmus, head and yaw head tilt, bouts of spontaneous barrel rolling and circular walking. The animals were allowed to recover for either 48 h or 7–10 days after surgery, at which point they were anaesthetized with halothane and decapitated with a small animal guillotine. The brain removed into ice-cold artificial cerebrospinal fluid (aCSF; composition in (mM): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$ and 10 D-glucose equilibrated with 95% O_2/5% CO2) for the preparation of the slices of the MVN [9]. The brain stem extending from the inferior colliculus to the obex was isolated and cemented with the fourth ventricle uppermost to the stage of a Vibroslice (Campden Instruments, Loughborough, UK). Horizontal slices of 350 μm containing the MVN were cut. Each slice was cut down the midline to give two isolated medial vestibular nuclei. Care was taken to unambiguously identify the left and right nuclei (the ipsilesional and contralesional MVN respectively), which were transferred separately to an interface-type incubation chamber that was continuously perfused with aCSF equilibrated with 95% O_2/5% CO_2 (flow rate 1.8 ml min^{-1}), and maintained at 33 ± 0.2°C. After an incubation period of 1 h, conventional glass micropipettes filled with 2 M sodium gluconate (impedance 5–8 MΩ) coupled to an Axoclamp 2B amplifier (Axon Instruments, USA) were used to systematically explore the rostral and caudal areas of each MVN for tonically firing neurones [4,5]. The spontaneous discharge rate of tonically active MVN neurones was displayed and analysed online using a micro 1401 interface (CED, Cambridge, UK) linked to a PC running Spike 2 software (CED, Cambridge, UK).

The $GABA_A$ agonist muscimol (5-aminomethyl-3-hydroxyisoxazole) and $GABA_B$ agonist baclofen (4-amino-3-[4-chlorophenyl]-butanoic acid) were obtained from Sigma (Dorset, UK). Aliquots (500 μl) of stock solutions of the drug were made up in distilled water and frozen until used. Test solutions of each agonist were made up by diluting the stock solution in oxygenated aCSF immediately before use, and applied to the slices by switching the perfusion inlet to a reservoir containing the agonist. The inhibitory response of each cell to a 60 s test pulse of each agonist was measured as the maximal decrease in discharge rate expressed as a percentage of the resting discharge rate [9]. Data were analysed using SigmaStat for Windows (Jandel Scientific, Germany). Values are expressed as mean ± SEM. These experiments were carried out in accordance with current legislation on animal experimentation in the UK.

RESULTS

Increase in intrinsic excitability of rostral ipsilesional MVN cells after UVD: In an initial experiment, we made extracellular recordings of the tonic *in vitro* discharge rates of MVN cells in the rostral and caudal regions of the ipsilesional nucleus, in slices prepared from animals that had been labyrinthectomized either 48 h or 7–10 days beforehand. The mean resting discharge rates of the MVN cells at these time-points, together with the normal controls and data obtained in 4 h and 24 h post-UL slices from our earlier study [4], are shown in Fig. 1. As we reported previously the mean *in vitro* firing rate of rostral, but not caudal, ipsilesional MVN cells is significantly higher than control at 4 h and 24 h post-UL. As shown in Fig. 1, this increased excitability of the rostral MVN neurons was also seen in slices prepared from animals that had compensated for 48 h and 7–10 days post-UL (48 h post-UL, mean 18.6 ± 1.5 spikes s^{-1}, $n = 42$ cells; 7–10 days post-UL, mean

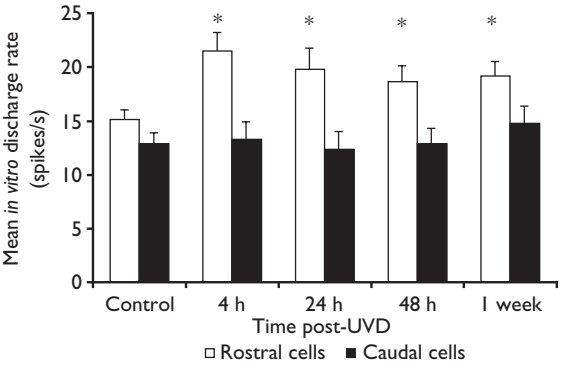

Fig. 1. Up-regulation of intrinsic excitability of rostral ipsilesional MVN neurones after unilateral labyrinthectomy. Mean (± s.e.m.) spontaneous *in vitro* discharge rates of MVN neurones in the rostral and caudal regions of the ipsilesional MVN, recorded extracellularly in slices prepared from animals that had been labyrinthectomised 4 h, 24 h, 48 h and 7–10 days beforehand. Asterisks indicate significant differences from control ($p > 0.05$, Mann Whitney rank sum test). The control, 4 h and 24 h data are from an earlier study [4].

19.7 ± 1.1 spikes s^{-1}, $n = 40$ cells; control, mean 13.7 ± 1.0 spikes s^{-1}, $n = 25$ cells, $p < 0.05$, Mann-Whitney rank sum test). The *in vitro* discharge rate of caudal ipsilesional MVN cells did not show significant changes compared with controls at any time-point post-UL (Fig. 1).

Responsiveness of ipsilesional and contra-lesional MVN cells to muscimol and baclofen: The inhibitory effects of the $GABA_A$ agonist muscimol and the $GABA_B$ agonist baclofen on the spontaneous resting discharge of rostral MVN neurons in the ipsilesional and contra-lesional nuclei were investigated, in slices prepared from animals that had compensated for 7–10 days after UL. In slices from normal animals, bath application of muscimol (1–10 μM, $n = 12$ cells) and baclofen (0.6–10 μM, $n = 13$ cells) caused a reversible, dose-dependent inhibition of the tonic discharge of all MVN cells tested, in a similar way to that reported earlier [6,9] (see Fig. 2a). In slices prepared from animals that had compensated for 7–10 days post-UL, the responsiveness of ipsilesional and contra-lesional MVN neurons to muscimol was not different from controls (Fig. 2b left panel, $n = 19$ cells in seven slices and $n = 16$ cells in seven slices, respectively). By contrast, the inhibitory response of ipsilesional rostral cells to baclofen was significantly lower than control at each dose tested, and there was a rightward shift of the dose–response relationship (Fig. 2b, right panel, $p < 0.05$ ANOVA on ranks, $n = 21$ cells in nine slices). The responsiveness of contralesional MVN neurons to baclofen was not different from controls (Fig. 2b right panel, $n = 19$ cells in seven slices).

DISCUSSION

These results show first that the up-regulation of intrinsic excitability that is observed in rostral ipsilesional MVN

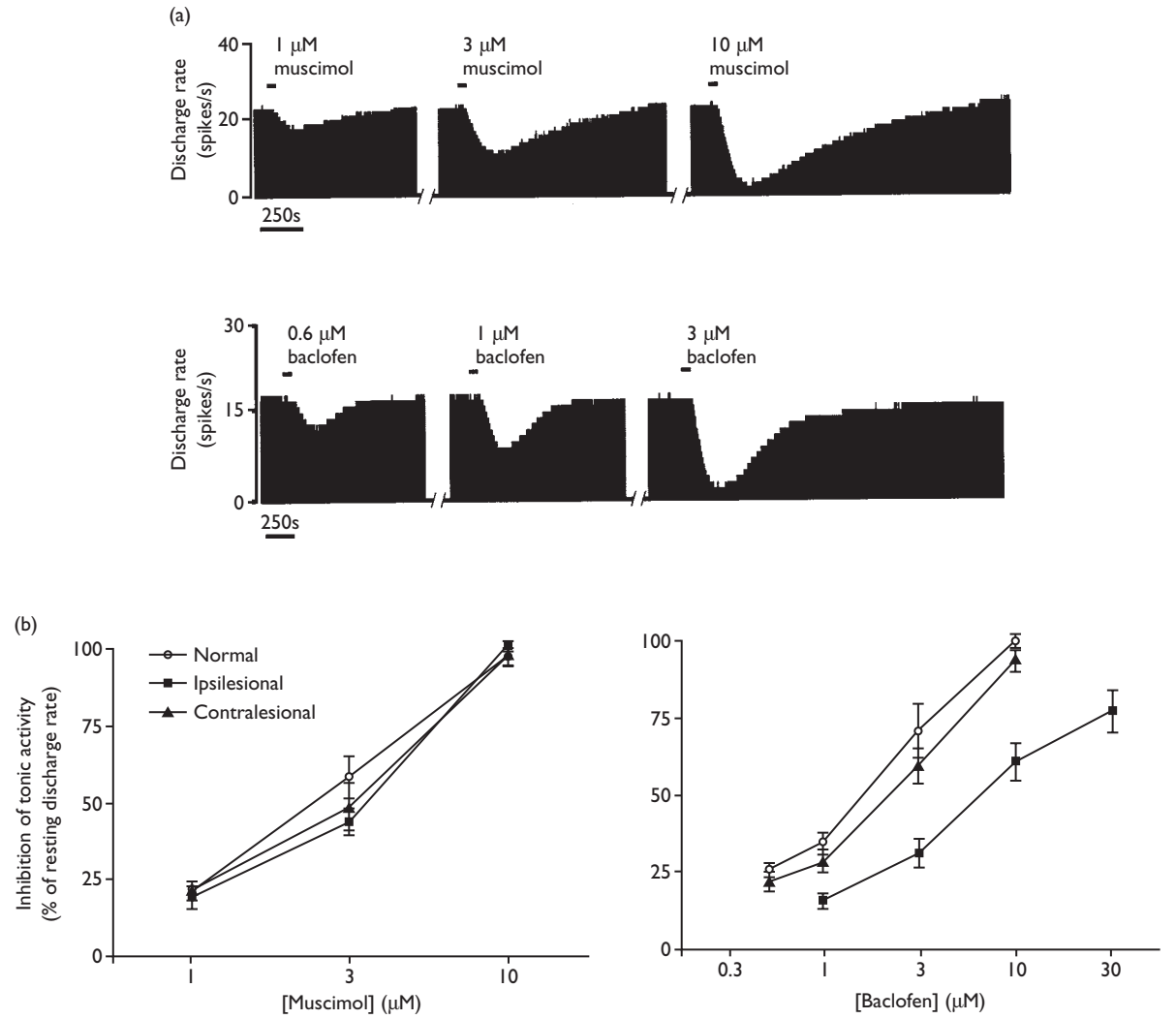

Fig. 2. Responses of ipsilesional and contralesional rostral MVN neurons to the $GABA_A$ agonist muscimol and the $GABA_B$ agonist baclofen *in vitro*. (**a**) Example firing rate histograms showing the inhibitory effects of muscimol (upper panel) and baclofen (lower panel) on the tonic activity of two representative MVN cells. The 60 s periods of bath application of the agonists are indicated by the bars above the data. (**b**) Dose–response relationship of the inhibitory responses to muscimol (left panel) and baclofen (right panel) on rostral MVN neurons in control slices (open symbols), and in ipsilesional and contralesional slices (filled symbols) from animals after 7–10 days of compensation after UL.

neurons within 4 h post-UL [4,5], persists in slices from animals that have compensated for 7–10 days post-UL. In our initial study [4] we measured the *in vitro* firing rates of ipsilateral and contralesional, rostral and caudal MVN cells at various times between 2 and 48 h after UVD, and our sample sizes in each case were consequently relatively small. In that study, the mean *in vitro* discharge rate of the ipsilesional rostral cells was found to be higher than control at 48 h post-UL, but this difference was not sufficient to be statistically significant. In the present study we concentrated on the 48 h and 7–10 day time-points post-UL, and showed that the mean firing rates of the ipsilesional rostral cells remain significantly higher than normal for up to at least 7–10 days post-UL (Fig. 1). Thus the up-regulation of intrinsic excitability in the ipsilesional MVN cells is not a transient phenomenon that follows UL, but instead it is a sustained adaptive change in their electrophysiological properties that occurs soon after deafferentation and persists through and beyond the period over which the early behavioural recovery takes place.

Second, these results show that $GABA_A$ and $GABA_B$ receptors in the ipsilesional MVN neurons have distinct roles to play in the induction and maintenance of vestibular compensation after UL. In the short term, there is a rapid down-regulation of the functional efficacy of both $GABA_A$ and $GABA_B$ receptors in the ipsilesional neurons [6]; together with the up-regulation of intrinsic excitability, this presumably helps to bring about the recovery of resting activity in these cells by counteracting the disfacilitation and excessive commissural inhibition which silences them after UL *in vivo*. In the longer term however, the present results show that the functional efficacy of $GABA_A$ receptors returns to normal within 7–10 days post-UL, while the efficacy of $GABA_B$ receptors remains significantly down-regulated. The recovery of fast $GABA_A$ mediated neurotransmission presumably enables the ipsilesional cells to again respond to vestibular stimulation and participate in vestibular information processing, through commissural inhibitory modulation from the intact side. Simultaneously however the permanent loss of excitatory input from the lesioned primary vestibular afferents is, in effect, counteracted by a sustained down-regulation of the efficacy of metabotropic $GABA_B$ receptors. This reduction in responsiveness of the deafferented neurons to inhibitory inputs may enable their intrinsic pacemaker-like membrane conductances [10,11] and other exciatory synaptic inputs to maintain their resting discharge in the long term.

Our results are in line with the finding of Calza *et al*. [12], who found that the density of $GABA_A$ receptors as assessed by benzodiazepine binding in the ipsilesional MVN was significantly reduced 24 h post-UL but had returned to normal by 3 days. Our findings also provide a potential explanation at the cellular level for the effects of the $GABA_B$ receptor agonist baclofen and the antagonist CGP 36742 on the VOR in long-term compensated animals reported recently by Magnusson *et al*. [13,14]. Thus, Magnusson *et al*. [13] showed that systemic administration of baclofen, but not the $GABA_A$ agonist THIP, dose-dependently reduced or abolished the residual asymmetry in horizontal VOR gain in rats that had compensated for 8–12 weeks post-UL. In addition, the $GABA_B$ receptor antagonist CGP 36742 caused the reappearance of vestibular symptoms (de-compensation) in animals that had compensated for 4 weeks post-UL [14]. These effects may be explained by a persistent, long-term down-regulation of $GABA_B$ receptor efficacy in the ipsilesional MVN neurons, so that baclofen and CGP 36742 given systemically act predominantly on the contralesional MVN neurons but have little effect on the ipsilesional MVN neurons. Thus, systemic baclofen administered to a compensated animal can be expected to inhibit the firing rate of contralesional neurons but have a relatively much weaker effect on ipsilesional neurons, whose $GABA_B$ receptor efficacy is significantly down-regulated. Similarly the $GABA_B$ antagonist CGP 36742 is likely to disinihibit contralesional MVN neurons by partly relieving them of GABAergic inhibitory tone, but have relatively little effect on ipsilesional cells. The lack of similar effects of THIP may be explained by the fact that at 7–10 days post-UL there is no longer a difference in $GABA_A$ receptor efficacy in the MVN neurons of the lesioned and intact sides. The finding of Magnusson *et al*. [13] that the effects of baclofen on the VOR persist in animals that have compensated for up to 3 months after UVD, indicates that $GABA_B$ receptor efficacy in ipsilesional neurons remains down-regulated in the long-term, perhaps permanently.

These results therefore provide the first evidence for a differential adaptive regulation of the functional efficacy of $GABA_A$ and $GABA_B$ receptors during CNS plasticity, and they indicate a novel and fundamental role for $GABA_B$ receptors in the long-term maintenance of the behaviourally compensated state after unilateral vestibular deafferentation.

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