Research report

The effects of protein kinase C and calmodulin kinase II inhibitors on vestibular compensation in the guinea pig

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Abstract

Previous studies have demonstrated that vestibular compensation, the process of behavioural recovery which occurs following unilateral deafferentation of the vestibular labyrinth (UVD), is correlated with changes in in vitro phosphorylation of various protein substrates in the brainstem vestibular nucleus complex (VNC). The aim of the present study was to investigate the possible causal relationship between protein kinase activity and the induction of the vestibular compensation process, by delivering inhibitors of protein kinase C (PKC) or Ca2+/calmodulin-dependent kinase II (CaMKII) into the ipsilateral VNC at the time of the UVD and determining their effects on three static symptoms of UVD, spontaneous nystagmus (SN), yaw head tilt (YHT) and roll head tilt (RHT) in guinea pigs. Infusion of the PKC inhibitor, 3-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione, HCl (bisindolylmaleimide I, HCl / GF 109203X, HCl) (‘Bis I’), at a concentration of 5 or 50 μM, significantly increased SN frequency at the earliest time points (6 and 8 h post-UVD) compared to vehicle controls and the less selective analogue, 2,3-bis(1H-indol-3-yl)-N-methylmaleimide (bisindolylmaleimide V) (‘Bis V’). However, the compensation of YHT and RHT was unaffected by the PKC inhibitor. By contrast, the cell-permeable CaMKII inhibitor, myristoylated autocamtide-2 related inhibitory peptide (N-Myr-Lys-Lys-Ala-Leu-Arg-Arg-Gln-Glu-Ala-Val-Asp-Ala-Leu-OH) (‘myr-AIP’) or the cell-impermeable analogue, autocamtide-2 related inhibitory peptide (N-Lys-Lys-Ala-Leu-Arg-Arg-Cln-Glu-Ala-Val-Asp-Ala-Leu-OH) (‘AIP’), failed to alter the compensation of SN, YHT or RHT at any dose compared to vehicle controls. These results implicate PKC-, but not CaMKII-, signal transduction pathways in the initiation of SN compensation in guinea pig. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Sensory systems

Topic: Auditory, vestibular, and lateral line: periphery

Keywords: Protein kinase C; Vestibular compensation; Ca2+/calmodulin-dependent kinase II; Vestibular nuclei; Neural plasticity

1. Introduction

Unilateral deafferentation of the peripheral vestibular labyrinth (UVD) results in a complex syndrome of eye movement and postural disorders which arises from the imbalance in tonic and dynamic neuronal activity in the vestibulo-ocular and vestibulo-spinal reflex pathways [43]. The static symptoms of UVD have been referred to as those which persist in the absence of head movement [e.g., spontaneous nystagmus (SN), yaw head tilt (YHT) and roll head tilt (RHT)] and therefore relate to the imbalance in resting activity between neurons in the ipsilateral and contralateral vestibular nucleus complexes (VNC). The dynamic symptoms are those which are observed during head movement [e.g., reduced gain of the vestibulo-ocular reflex (VOR)] and are related to the reduced sensitivity of VNC neurons to head movement stimuli (see Ref. [6] for a review). Over time, the severity of some static symptoms gradually decreases in a process of behavioural recovery known as ‘vestibular compensation’. Although a partial recovery of resting activity in the VNC ipsilateral to the UVD (ipsi-VNC) is believed to be largely responsible for

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the compensation of the static symptoms, the biochemical basis of this resting activity recovery, and the other neuronal changes responsible for compensation, are poorly understood (see Refs. [6,38] for a review).

Protein kinase C (PKC) and Ca\(^{2+}\)/calmodulin-dependent kinase II (CaMKII) are major serine/threonine protein kinases found in particularly high concentrations in brain [42]. There is good evidence that these kinases play important roles in several types of neuronal plasticity (e.g., [3,13]; see Ref. [40] for a recent review). A number of studies have shown that vestibular compensation in frog and guinea pig is accompanied by changes in the phosphorylation of specific brain proteins in vitro [10,22–24,35]. In guinea pig, the phosphorylation of at least one probable PKC substrate in the bilateral medial vestibular nucleus/prepositus hypoglossi, increased following compensation of the static symptoms compared to sham-operated controls [35]. This contrasts with a lack of Ca\(^{2+}\)/calmodulin-stimulated protein phosphorylation changes in the same tissue. There is also some evidence that the in vitro phosphorylation changes that occur in frog whole brains, removed at different stages of compensation, are mediated by an endogenous PKC and CaMKII [21,22]. These results suggest that the phosphorylation state of specific endogenous proteins in areas of the brain involved in processing vestibular information, is changed during vestibular compensation in vivo. Several potential mechanisms could account for these changes, including de novo synthesis of substrate proteins, and/or a change in kinase activity. Direct evidence that the initial stages of static compensation in guinea pig are not associated with a change in de novo protein synthesis was recently reported by Ris et al. [31], who found that systemic injections of a protein synthesis inhibitor (cycloheximide) had no effect upon the early stages of resting activity recovery in the ipsi-VNC of the guinea pig. However, the possibility that later stages of compensation might involve de novo protein synthesis cannot be excluded [4]. The hypothesis that mammalian vestibular compensation might involve a change in PKC activity is further supported by recent immunohistochemical experiments, which have shown that early stages of compensation in rat are accompanied by changes in the expression of vestibulo-cerebellum PKC-alpha, -gamma and/or -delta [2,12] or VNC PKC-delta [28]. Taken together, these data suggest that vestibular compensation of the static symptoms is correlated with changes in PKC/CaMK pathways in the VNC and/or cerebellum; however, it is not clear whether these changes are causally involved in the compensation process or merely coincident with it.

Despite the correlative evidence implicating protein phosphorylation in the VNC in the mechanisms of vestibular compensation [23,35], to date there has been only one study (in rat [1]) which has examined the effects of kinase inhibitors on the compensation process, and in this case a selective CaMKII inhibitor was not employed. The present experiment attempted to further investigate the role of protein kinases in static compensation by comparing the effects of PKC and CaMKII inhibitors, delivered into the ipsi-VNC at the time of the UVD in guinea pig.

2. Methods

2.1. Animals

Fifty-four adult male and female pigmented guinea pigs (Cavia porcellus; 290–725 g) were used in these experiments, twenty four each for the PKC inhibitor and CaMKII inhibitor experiments in which cannulae were placed stereotaxically in the right VNC (see Table 1). In addition, data for the two doses of the PKC inhibitor were obtained from six animals (n=3 for each dose) in which the cannula was placed in the IVth ventricle as an additional control. Although the animals used in these experiments varied in weight, they were all adult guinea pigs and in our previous studies we have not observed differences in vestibular compensation for adult animals of different weight [39].

2.2. Cannula implantation

All surgical procedures were approved by the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. The cannula implantation procedure has been described in detail elsewhere [34]. Briefly, animals were anaesthetised with fentazin (0.4 ml/kg, i.m.; fentazin contains 0.4 mg/ml fentanyl citrate, 58.3 mg/ml xylazine HCl and 3.2 mg/ml azaperone; Parnell Laboratories, Auckland) and a 21-gauge metal cannula was lowered through the cerebellar vermis into the right VNC.

Table 1

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<tr>
<th>Inhibitor</th>
<th>Concentration (µM)</th>
<th>Total dose (nmol)</th>
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<td>Bis I</td>
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<td>Bis V</td>
<td>5</td>
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<td>(5% DMSO in mACSF)</td>
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<td>CaMKII Inhibitors</td>
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<td>500</td>
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<td>AIP (cell impermeable)</td>
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complex (stereotaxic co-ordinates: 12.5 mm caudal; 1.5 mm lateral; 10.8 mm ventral to the skull surface) and secured to the skull with dental cement. Animals (n=6) with IVth ventricle placements were used as additional controls; in these animals the cannula had penetrated the cerebellum and there was clear evidence of dye infusion (see below) in that region, suggesting that the drug may have also diffused to some extent into the cerebellum. Some animals displayed transient SN and mild postural asymmetries during the first few hours after cannula implantation. As a necessary part of the cannula implantation procedure, some part of the cerebellum and VNC must be lesioned in order to position the cannula in the VNC. In our experience, transient vestibular symptoms indicate only a minor lesion and our previous studies indicate that such minor damage, while confirming that the cannula is in the right place, does not significantly affect vestibular function thereafter [5,34]. However, animals which failed to compensate for these symptoms in less than 24 h were excluded from the experiment.

2.3. Drugs

The PKC inhibitor 3-[(3-dimethylaminopropyl)-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione, HCl (bisindolylmaleimide I, HCII GF 109203X, HCl) and the less selective analogue, 2,3-bis(1H-indol-3-yl)-N-methylmaleimide (bisindolylmaleimide V), were purchased from LC Laboratories (USA). Bisindolylmaleimide I HCl (Bis I) is selective for PKC in the nM range in vitro ([IC50=31 nM] [41]) but will inhibit other protein kinases at much higher concentrations; for example, the IC50's of Bis I for phosphorylase kinase, PKA, the endothelial growth factor receptor and the insulin receptor are: 0.7 μM, 2 μM, >100 μM, >100 μM, respectively [26,41]. In contrast, the affinity of bisindolylmaleimide V (Bis V) for PKC is greatly reduced (IC50>100 μM) [8] and was used as a negative control for possible non-specific effects of Bis I. The doses of Bis I were chosen based upon data from from 4.21 to 4.34 μM, being placed inside the guide cannula in the animal. The pseudo-substrate CaMKII inhibitors, myristoylated autocamtide-2 related inhibitory peptide (myr-AIP; N-Lys-Lys-Ala-Leu-Arg-Arg-Gln-Glu-Ala-Val-Asp-Ala-Leu-OH; MW 1498), were purchased from Biomol Research Laboratories (USA). These peptides are analogs of the synthetic CaM-KII substrate, autocamtide-2. myr-AIP is reputedly cell-permeable due to the addition of a myristoyl group (a saturated fatty acid) on the N-terminus [16,19,20]. Since AIP lacks this myristol group it was used as a cell-impermeable control. The concentrations of AIP used in the present experiments were based upon those used in an in vitro phosphorylation assay of post-synaptic density proteins [15], since to the best of our knowledge, there are no published reports of its use in vivo. Each peptide was reconstituted in sterile filtered millipure water to a stock solution of 1000 μM, and stored as frozen aliquots at −80°C for a maximum of 1 month to ensure optimal stability. On the day of each experiment, frozen aliquots were thawed at room temperature and diluted to working concentrations with sterile mACSF (pH 7.0). Vehicle controls for both the Bis (5% DMSO in mACSF) and AIP (mACSF) groups were run in the same way as the experimental groups (see Table 1).

2.4. Drug delivery

Guinea pigs were randomly assigned to one of four experimental groups (see Table 1). Two 25-μl Hamilton syringes were attached to a 1m length of polyethylene tubing and placed into a syringe pump (Harvard Instruments, MA, USA). Distal to the syringes, each infusion line was attached to an infusion needle constructed of 27-gauge stainless steel tubing. Drug or vehicle solutions were backfilled into the tubing prior to the infusion needle being placed inside the guide cannula in the animal. The syringe pump delivered drug solutions at a constant rate of 2 μl/h for a total of 2 h, beginning 1 h pre-, and finishing 1 h post-UVD. The total infusion volume was verified after each infusion. The group mean infusion volumes ranged from 4.21 to 4.34 μl. Each animal was given a single dose of the sedative/muscle relaxant, xylazine (12 mg/kg, i.m.), 10 min prior to the start of the cannula infusion. Approximately 30 min after the start of the infusion, animals were anesthetised for UVD surgery. For PKC inhibitor experiments, all drug and vehicle solutions were administered blind; solutions were coded by a third party and codes were not revealed to the experimenter until after the experiments were completed. Drug and control conditions were run together on the same day where possible. CaMKII inhibitor experiments were not run blind because AIP peptides have a limited shelf-life in solution (approximately 1 month at −80°C), therefore control and experimental conditions could not be run in parallel.

Since parts of the VNC border on the IVth ventricle, we took the following precautions to minimise drug spread from the injection site into the IVth ventricle, surrounding brainstem nuclei and cerebellum. First, the slow rate of
drug infusion (2 μl/h) means that drug concentrations at sites distant from the target nuclei will be low compared to the injection site [36,44]. The infusion rate used in this study is comparable to that of some osmotic minipumps. Second, the diameter of the injection needle and inner cannula were identical in order to minimise drug backflow up the sides of the injection needle.

2.5. Unilateral vestibular deafferentation (UVD)

One week following cannula surgery, all animals were given a right global surgical labyrinthectomy (UVD), as described in detail elsewhere [34]. Briefly, guinea pigs were anaesthetised and prepared for surgery in an identical manner to that described for the cannula surgery above. A horse-shoe-shaped incision was made over the right ear and the temporal bone exposed following blunt dissection of the temporalis muscle and its subsequent removal with surgical scissors. The bony labyrinth was exposed with a high speed drill, the horizontal and anterior semicircular canal ampullae and otoliths were subsequently destroyed under microscopic control using a fine burr, and their contents aspirated. Our previous studies have shown that this UVD procedure results in complete destruction of the vestibular receptors [5,11,23,33–35,39].

2.6. Behavioural measurements

Spontaneous nystagmus (SN), roll head tilt (RHT) and yaw head tilt (YHT) were measured for each animal at 6, 8,10, 12, 20, 25, 30, and 50 h post-UVD. Measurements could not be carried out earlier than 6 h post-UVD due to the possibility of lingering anesthetic affects. All behavioural measurements for experiments using PKC inhibitors were carried out double-blind. SN, YHT and RHT were measured using video techniques and the procedures we have described in detail previously (e.g., [5,11]). Mean SN, YHT and RHT were calculated for each measurement time within each group of animals.

2.7. Statistical procedures

All data were analysed using a three factor analysis of variance (ANOVA) with repeated measures using the statistical software package SPSS (v6.1.1). Drug and dose represented between-subjects factors while time represented the within-subjects factor (i.e. the repeated measure). A significant drug or dose interaction with time represented evidence of a drug or dose effect on the rate of compensation. Analysis of between-group simple main effects was carried out using a standard two factor ANOVA (drug and dose) at the first two time points (alpha=0.05) [17]. Only data from the 5 and 50 μM doses of Bis I and V or vehicle were subjected to statistical analysis since the 200 μM Bis solutions contained a higher concentration of DMSO (i.e., 10% cf. 5% for lower doses). In addition, no statistical comparisons were made between Bis I and V at the 200 μM dose, because of the small sample sizes involved (n=2 per group).

2.8. Histology

Cannula tip positions were verified for each animal in these experiments. At the conclusion of the experiment, animals were given an overdose of pentobarbital followed by a 0.5–1.0 μl cannula injection of dye (Alcian blue 8GX, 5% w/v) to mark the infusion site. Animals were then transcardially perfused with 0.9% saline followed by a phosphate-buffered formaldehyde (10%) solution. The brainstem and cerebellum were removed and stored in a formaldehyde solution (see above) until sectioned. The fixed tissue was mounted on a microtome stage, frozen with CO₂ and 80–90 μm coronal sections cut serially with a Leitz microtome. The coronal sections were mounted on gelatin-coated slides and air dried for 24 h before staining with thionin blue and coverslipping. At least one slide from each animal was photographed (Kodak Ektachrome 64T film).

3. Results

3.1. Histological verification of cannula tips

Alcian blue dye spots were used to mark the injection site and hence the position of the cannula tip, but were not intended to measure drug spread from the injection site. Cannula tips of all animals were located within the ipsi-VNC or on its border (Fig. 1A–B) or in the IVth ventricle in the case of the additional control animals described above (data not shown).

3.2. Spontaneous nystagmus compensation

Animals treated with the PKC inhibitor Bis I (5 or 50 μM), had significantly higher levels of SN compared to controls (Bis V or vehicle groups) (F(1, 15)=6.70; P<0.05; Fig. 2A–B). Analysis of simple main effects showed that the Bis I-induced increase in SN was significantly different from controls only at 6 and 8 h post-UVD (F(1, 15)=9.77; P<0.05). These times correspond to 5 and 7 h following Bis I infusion. Following this, SN frequencies began to decrease and became similar to those of controls by approximately 20 h post-UVD, and remained this way throughout the course of compensation (Fig. 3A–B). The 50 μM dose of Bis I produced the greatest increase in SN at 8 h post-UVD; 87% and 88% higher than Bis V and vehicle groups, respectively (Fig. 3B). Although 50 μM Bis I resulted in higher mean SN across all time points compared to the 5 μM dose (Fig. 3A c.f. 3B), this difference was not statistically significant. There were no significant drug or dose interactions with time, suggesting
that neither Bis I or Bis V, at 5 or 50 μM, altered the rate of SN compensation. Importantly, there was no significant difference in SN compensation between Bis V (5 or 50 μM doses) and vehicle groups, indicating that the less potent PKC inhibitor Bis V, had no effect on the compensation process. These results contrast with the obvious lack of difference in SN between 200 μM Bis I and Bis V at any time point (Fig. 2C), although the data were not subjected to statistical analysis (see Methods).

Detection of the quick phase of SN was difficult in some animals at early measurement times (i.e. 8/24 animals at 6–12 h in the Bis I, V and Bis vehicle groups) and was probably due to a reduction in the amplitude of the quick phase. Fewer animals (4/24) showed this effect up to 30 h post-op. It is therefore possible that SN frequency was underestimated on these occasions early in the compensation process. However, since the effect was present in only a subset of animals from both the drug and control groups, it seems unlikely that an underestimation of SN frequency in these animals could account for differences in SN between groups. The possible reduction in quick phase amplitude was not due to an action of DMSO per se, since: (1) not all animals were affected; (2) the magnitude of the effect did not change with increasing DMSO concentration (the vehicle for the 200 μM dose condition contained 10% DMSO c.f. 5% in other groups); (3) there were no significant differences in compensation of SN between animals infused with a vehicle containing 5% DMSO in mACSF and mACSF alone (data not shown). VOR/okotoxinetic reflex eye movements could be evoked in all of these animals by rotation in light. One possible explanation is that the effect was due to a combination of DMSO and
Fig. 2. Effect of 5 μM (A), 50 μM (B) or 200 μM (C) of either the selective PKC inhibitor (Bis I), the less selective PKC inhibitor (Bis V), or vehicle on the compensation of spontaneous nystagmus (SN). Symbols represent means ± S.E. When 5 and 50 μM dose groups were combined, the difference between Bis I and Bis V at 6 and 8 h post-UVD was statistically significant (P < 0.05). Bar represents the drug infusion period.

Fig. 3. The change in spontaneous nystagmus (SN) frequency expressed as % of vehicle (100%) produced by either 5 μM (A) or 50 μM (B) Bis I or Bis V over time post-UVD. Symbols represent the ratio of two means. When 5 and 50 μM dose groups were combined, the difference between Bis I and Bis V at 6 and 8 h post-UVD was statistically significant (P < 0.05).

Fig. 4. Infusion of the CaMKII inhibitor, myr-AIP, into the ipsi-VNC at the time of the UVD had no significant effect upon the compensation of SN, at doses ranging from 10 to 500 μM (Fig. 4).

3.3. Postural compensation

In contrast to SN, there were no significant differences in the compensation patterns of RHT or YHT between animals treated with the PKC inhibitor, Bis I (5 or 50 μM) and controls (data not shown). As has been reported in numerous other studies, the compensation of RHT and YHT within groups was extremely variable [9,33,34,37,39].

A number of animals in both the mAIP (2/11 animals) and AIP (5/10) groups, and one animal (1/3) in the vehicle group, could not be measured for either RHT or YHT at early time points because they could not stand unassisted. Since a greater number of animals in the AIP group were affected, statistical comparisons were made between mAIP and vehicle (mACSF) data only, beginning at 10-h post-UVD. There were no statistically significant differences in RHT or YHT between myr-AIP (at any dose) and vehicle groups (data not shown).

4. Discussion

The results of the present experiment show that infusion of a PKC inhibitor into the ipsi-VNC at the time of the UVD, increased mean SN frequency between 6 and 8 h
possibility that the behavioral effects observed were due to drug action on the VNC. The exact mechanism(s) by which Bis I produced its effects upon SN frequency cannot be determined from the present experiment. It is possible that Bis I affected SN indirectly, for example, by blocking one or more components of the anaesthetic (fentanyl), resulting in a greater expression of behavioural symptoms at early time points. However, this seems unlikely given that: (1) only SN was affected and not postural symptoms; (2) animals showed no obvious signs of sedation at the earliest measurement time (6 h post-UVD); and (3) similar results have recently been obtained with Bis I using a different anaesthetic in a different species [2].

As many previous reports have shown, RHT and YHT are highly variable in mammals (9,29,32,34,37,39; see Ref. [6] for a review). However, it seems unlikely that the lack of statistically significant effects on posture shown in the present study was simply due to the inherent variability since a number of laboratories (including our own) have reported statistically significant drug effects on postural compensation in guinea pig [5,18,27,33]. This finding is consistent with the idea that the induction of postural compensation in guinea pig does not involve PKC or CaMKII activity in the ipsi-VNC.

During completion of the present study, Balaban et al. [2] reported the effects of various serine/threonine protein kinase inhibitors on the induction of SN compensation in male Long–Evans rats. Each rat was given a single intraventricular bolus injection of one of the following kinase inhibitors: Bis I (10 μM), A-3 (inhibits cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG; 1 mM)), H-7 (a non-selective inhibitor of PKC, PKA and PKG; 1 or 10 mM), Iso-H-7 (an inhibitor of PKC and cyclic nucleotide-dependent protein kinases; 10 or 50 mM), or distilled water (control). Injections were given 15 min pre-UVD. SN frequency was post-UVD compared to controls. However, the compensation patterns of RHT and YHT were unaffected by the PKC inhibitor. In contrast, the cell-permeable CaMKII inhibitor (myr-AIP) failed to alter the compensation pattern of SN, RHT or YHT, compared to controls. These results show that inhibition of PKC, but not CaMKII, in the ipsi-VNC changes the pattern of SN compensation in guinea pig, and suggest that a causal link may exist between PKC activity and the initiation of SN compensation. It should be noted that while Bis I affected the compensation of SN at early time points, it did not prevent its compensation altogether. This suggests that PKC might not be the only signalling pathway involved.

Despite our attempts to limit drug administration to the VNC, it is probably inevitable that some drug would have spread beyond the injection site into surrounding areas (if only by simple diffusion). This possibility is supported by the fact that IVth ventricle infusions of Bis I produced similar effects to infusions of the same drug into the ipsi-VNC. In either case, however, one cannot exclude the possibility that the behavioral effects observed were due to drug action on the VNC.

The need to use relatively high concentrations of kinase
inhibitors in vivo has the potential to limit their selectivity, and therefore, it is possible that the effects of Bis I in the present study were due to inhibition of kinases other than PKC and/or non-specific actions. The following evidence suggests that this was not the case. First, the behavioural effects produced by Bis I were different to those produced by the same dose of the less potent analogue, Bis V, or the cell permeable CaMKII inhibitor, myr-AIP. Second, the effects of Bis I showed a trend toward dose-dependency. Both of these factors suggest that Bis I was producing its effects by binding to a specific receptor, probably PKC, rather than having a non-specific action. Third, protein phosphorylation changes which have been demonstrated in the guinea pig medial vestibular nucleus/prepositus hypoglossi in vitro following UVD, were probably mediated by an endogenous PKC rather than CaMKII, PKA or PKG [23,35]. Furthermore, in the Balaban et al. [2] study, the increased SN produced by the non-selective protein kinase inhibitor, H-7, occurred over a different time course compared to that observed following Bis I in rat. Fourth, it seems unlikely that the behavioural effects of Bis I in the present experiment can be explained by PKA or PKG inhibition (despite the fact that the IC₅₀ of Bis I for PKA is relatively low, 2 μM in vitro), for the following reasons: (a) Balaban et al. [2] have shown that a PKA and PKG inhibitor had no effect on SN compensation in rat; (b) the endogenous activities of PKA and PKG in the guinea pig medial vestibular nucleus/prepositus hypoglossi [23,35] are particularly low compared to other brain areas; (c) biochemical experiments have shown that 5 μM Bis I did not affect cAMP-dependent phosphorylation in vitro [41].

The transient nature of the behavioural effects produced by Bis I and the fact that it affected only one UVD symptom—SN—suggest that its effects were not due to toxicity. Furthermore, all of the animals receiving Bis I compensated to the same extent and within the same time period as the control group. There was no behavioural evidence of ill-health in the Bis-treated animals and histology did not indicate any obvious toxic effects on the morphology of VNC neurons. Finally, if the effect of Bis I on SN was due to toxicity, it would be expected that higher doses would produce even greater behavioural effects; however, the effect of the highest dose of Bis I (200 μM) was not significantly different to the vehicle control.

We know of no other studies in which the CaMKII inhibitors myr-AIP or AIP have been used in vivo. Consequently, it is important to note that the in vivo mechanism of drug action is not necessarily the same as that in cell-free, or whole cell preparations in vitro. Therefore, the conclusion that CaMKII might not be involved in static compensation must be a tentative one at this stage.

It is unclear precisely how PKC-mediated signalling pathways in the ipsi-VNC may contribute to the initiation of SN compensation. Potential mechanisms could involve a change in the intrinsic properties of ipsi-VNC neurons, such as modulating ion channels involved in generating pacemaker activity [7]; see Ref. [30] for contrary evidence) or a change in the efficacy of synaptic inputs which could involve, among other things, a change in transmitter release (see Ref. [25] for a recent review). Interestingly, Qian and Barmack [28] have shown a decrease in the transport of PKC-delta to Purkinje cell axon terminals within the ipsilateral medial vestibular nucleus and prepositus hypoglossi following UVD in rat. The authors speculated that these changes might contribute to compensation by reducing GABA release onto the ipsi-VNC, thereby disinhibiting the spontaneous firing rate of these neurons. This hypothesis is consistent with the results of the present experiment, in that PKC-delta inhibition by Bis I would be expected to prevent this disinhibition, resulting in the exacerbation of UVD symptoms.

Finally, it will be important for future experiments to confirm that the effects produced by Bis I were mediated through PKC; for example, whether the increase in SN can be reversed by acute administration of PKC activators, and/or mimicked by chronic administration of PKC activators through down-regulation of endogenous PKC activity. The use of antisense oligonucleotides to ‘knock-down’ specific PKC isozymes in the VNC, as has already been used successfully elsewhere, may also assist in identifying which isozymes are involved in these effects.

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