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CHARACTERIZATION OF HISTAMINE PROJECTIONS AND THEIR POTENTIAL CELLULAR TARGETS IN THE MOUSE RETINA

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Abstract—The vertebrate retina receives histaminergic input from the brain via retinopetal axons that originate from perikarya in the posterior hypothalamus. In the nervous system, histamine acts on three G-protein-coupled receptors, histamine receptor (HR) 1, HR2 and HR3. In order to look for potential cellular targets of histamine in the mouse retina, we have examined the retina for the expression of histamine and the presence of these three receptors. Consistent with studies of retina from other vertebrates, histamine was only found in retinopetal axons, which coursed extensively through the ganglion cell and inner plexiform layers. mRNA for all three receptors was expressed in the mouse retina, and immunohistochemical studies further localized HR1 and HR2. HR1 immunoreactivity was observed on dopaminergic amacrine cells, calretinin-positive ganglion cells and axon bundles in the ganglion cell layer. Furthermore, a distinct group of processes in the inner plexiform layer was labeled, which most likely represents the processes of cholinergic amacrine cells. HR2 immunoreactivity was observed on the processes and cell bodies of the primary glial cells of the mammalian retina, the Müller cells. This distribution of histamine and its receptors is consistent with a brain-derived source of histamine acting on diverse populations of cells in the retina, including both neurons and glia.

Key words: histamine, histamine receptors, mouse retina, immunohistochemistry.

Visual processing begins in the retina. Here, contrast, color, shape and movement of an object are analyzed and processed in discrete circuits and pathways. The processed primary information is then sent to the brain via the output cells of the retina, the ganglion cells. In contrast to this well-established visual pathway from eye to brain, projections from the brain to the retina (retinopetal) have also been described. Indeed, Cajal identified these fibers in avian species more than one century ago (Ramón y Cajal, 1889). In fish, the retinopetal axons project from the olfactory bulb and appear to have a function linking olfaction with vision (Maaswinkel and Li, 2003). However, the function of these retinopetal projections in all other animals has not been determined.

Retinopetal projections from the brain have been described in most mammals including man (Repérant et al., 1989). A series of immunohistochemical studies indicates that a major proportion of retinopetal axons in primate, rat and guinea pig contain histamine, and that they arise from the tuberomammillary nucleus in the posterior hypothalamus (Airaksinen and Panula, 1988; Panula et al., 1989; Labandeira-Garcia et al., 1990; Gastinger et al., 1999, 2001). These studies also indicated that there are no histamine immunoreactive cell bodies in the retina, suggesting that the brain is the sole source of histamine for the retina.

In the mammalian brain, histamine producing neurons are confined to the tuberomammillary nuclei and innervate essentially all areas of the brain, including the cerebral cortex, amygdala, basal ganglia, hippocampus, and thalamus, as well as the retina (Haas and Panula, 2003). The brain histamine system contributes to the ascending arousal system, which is involved in diverse activities including waking, feeding and drinking, sexual activity, novelty and arousal/stress states associated with fear and danger (Schwartz et al., 1991; Blanco et al., 2001; Haas and Panula, 2003). Mice deleted for either the histamine producing enzyme histamine decarboxylase (HDC), or for one of its receptors (histamine receptor 1, HR1), show deficits in both attention and interest in novel environments as well as subtle alterations in the sleep–wake cycle (Inoue et al., 1996; Yanai et al., 1998; Maccarrone et al., 2002). Further, histamine release throughout the brain varies with the behavioral state, being essentially off during sleep and high during arousal and stress (Haas and Panula, 2003). Histamine release in the retina is most likely regulated as it is in the brain and therefore it may have related functions associated with this arousal system.

The identification of the cells in the retina which express receptors for histamine would be very useful in identifying potential cellular targets for histamine action. In the CNS histamine acts on three G-protein-coupled receptors, HR1, histamine receptor 2 (HR2) and histamine receptor 3 (HR3) (Schwartz et al., 1991; Brown et al., 2001; Haas and Panula, 2003). A previous report has studied the expression of HR1 in the rat retina and of HR3 in the monkey retina (Gastinger et al., 2006). To date, however, a systematic analysis of all three receptors has not been undertaken in a single species.

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Abbreviations: BRB, blood–retinal barrier; CTA, Cas-Block, 0.3% Triton X-100, 0.3% sodium azide; dNTP, deoxyribonucleotide triphosphates; EDTA, ethylenediaminetetraacetic acid; GCL, ganglion cell layer; HDC, histamine decarboxylase; HR1, histamine receptor 1; HR2, histamine receptor 2; HR3, histamine receptor 3; INL, inner nuclear layer; IPL, inner plexiform layer; PB, 0.1 M sodium phosphate buffer, pH 7.4; RT, room temperature; RT-PCR, reverse transcriptase polymerase chain reaction; SDS, sodium dodecyl sulfate; TH, tyrosine hydroxylase.

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In the mouse, a large variety of different naturally occurring mutants and genetically modified strains is now available. Some of these strains have abnormal retinal phenotypes or have other advantages in the study of retinal function, and some stains affect histamine signaling (Yanai et al., 1998; Kobayashi et al., 2000; Ohtsu et al., 2001; Wilson et al., 2002; Dalke and Graw, 2005; Haverkamp et al., 2006). For this reason, the mouse is becoming increasingly recognized as a very useful model to study the mammalian retina. We have therefore investigated the presence of histamine and its receptors in the mouse retina. We undertook reverse transcriptase polymerase chain reaction (RT-PCR) of retinal mRNA using primers specific for HR1, HR2 and HR3. Moreover, we anatomically localized histamine and HR1 and HR2 expression in the retina using immunohistochemistry of whole mounts and sections of the retina. Further, the pattern of antibody labeling of the receptors by double-labeling with previously characterized, population-specific neuronal markers has been characterized.

EXPERIMENTAL PROCEDURES

Animals

Adult male and female C57 black mice were obtained from the University of Melbourne Animal Research Facility (Department of Anatomy and Cell Biology, University of Melbourne, Victoria, Australia). All animal experimentation was performed in accordance with the institutional animal experimentation ethics committee and with the ARVO Statement for the Use of Animals in Opthalmic and Vision Research. All efforts were made to minimize the number of animals used and their suffering. Mice were group housed under a normal 12-h light/dark cycle at 22±1 °C with *ad libitum* access to food and water.

Immunohistochemistry for histamine in the retina, brain and optic nerve

For immunohistochemical labeling of histamine, mice were deeply anesthetized with sodium pentobarbital and transcardially perfused with 0.1 M sodium phosphate buffer, pH 7.4 (PB) followed with ice-cold, freshly prepared 4% 1-ethyl-3-(3diethylaminopropyl)-carbodiimide in PB (Panula et al., 1989). Eyes were removed immediately, hemisected and the anterior eyecup removed with fine forceps. Posterior eye-cups were post-fixed overnight at 4 °C in the same fixative. Brains and optic nerve were dissected and post-fixed in the same way.

Whole retinae were isolated using fine forceps and cryoprotected in 10% sucrose in PB for 1 h followed by 20% sucrose for a further 1 h at room temperature (RT). Retinae were then frozen in isopentane pre-cooled in liquid nitrogen and thawed for two cycles to allow for optimal antibody penetration. Whole retinae were incubated in a blocking solution of Cas-Block (Zymed, San Francisco, CA, USA) with 0.3% Triton X-100 and 0.3% sodium azide (CTA) for 1 h at RT and then in primary antibody (polyclonal rabbit anti-histamine, Chemicon, Temecula, CA, USA) diluted 1:500 in CTA for 2 days; the first day at RT on an agitator, the second day at 4 °C with agitation. Retinae were then washed three times in PB and incubated in Alexa Fluor 488 donkey anti-rabbit IgG (Invitrogen, Mount Waverley, Victoria, Australia) diluted in 1:400 CTA for two nights at 4 °C. Whole retinae were washed in PB three times and mounted on glass slides. Whole-mount retinae were allowed to dry at RT until clear in appearance before coverslipping with DakoCytomation Fluorescent Mounting Medium (Dako, Botany, New South Wales, Australia).

Brains and optic nerve were cryoprotected in 10% sucrose in PB for 2 h at RT followed by 20% sucrose in PB for one night at 4 °C. Specimens were then immersed in Tissue Tek O.C. T compound (Sakura Finetek, Torrance, CA, USA) diluted 1:1 with 20% sucrose solution for 1 h, embedded in O.C.T. compound and frozen in isopentane pre-cooled in liquid nitrogen. Specimens were immediately transferred to a cryostat for temperature adjustment for 2–4 h at −20 °C. Brains were cut into 30 μm sections, which were allowed to dry at RT for 30 min, washed for 10 min in PB, then incubated in CTA for 1 h at RT. Primary antibody (anti-histamine) was then applied and sections were incubated for 24 h at RT and then at 4 °C for a further 24 h. Sections were washed twice in PB and then incubated in secondary antibody for 1 h at RT, washed twice in PB and coverslipped with DakoCytomation Fluorescent Mounting Medium. Optic nerve specimens were cut longitudinally into 30 μm sections and collected and processed for immunohistochemistry as free floating sections in the same way as the brain sections. Retinae, brains and optic nerve from histamine decarboxylase$^{-/-}$ mice (Ohtsu et al., 2001) were used as negative controls for histamine immunolabeling. Stainings were performed in the same way as described above and resulted in no specific staining above background (not shown).

Immunohistochemistry for HR1 and HR2

Mice were transcardially perfused with PB followed by 4% paraformaldehyde in 0.1 M PB. Eyes were immediately removed, hemisected and the lens and vitreous body removed with fine forceps. The posterior eyecups were post-fixed for 10 or 30 min in the same fixative. The specimens were cryoprotected in 10% sucrose in PB for 1 h at RT and then in 20% sucrose in PB overnight at 4 °C. Both differently fixed eyecups of each animal were then embedded together in O.C.T. compound and frozen in one block in isopentane pre-cooled in liquid nitrogen. Eye specimens were cut into 12 μm sections with a cryostat.

Some of the retinal specimens, which had been post-fixed for 30 min, were embedded in paraffin. For this they were dehydrated for 1 h in 70%; 90% and 3×100% ethanol, followed by 90 min in 50:50 histolene/ethanol; 100% ethanol and 2× histolene. After this they were incubated for 5 h in warm paraffin wax, which was changed every hour, before they were orientated and embedded into paraffin blocks and cooled. Eight micrometer paraffin sections were cut and de-waxed before staining. All paraffin sections (intended for anti-HR2e staining, see Table 1) and some of the cryostat sections intended for HR1 immunostaining were treated for antigen retrieval. For this, sections were boiled for 15 min in 0.1 M sodium citrate buffer, pH 6.0. They were then washed in PB and used for immunohistochemistry.

Cryostat and paraffin sections were blocked in CTA for 1 h at RT, after which primary antibody (see Table 1), diluted in CTA was applied. Sections were incubated in primary antibody for 24 h at RT and then at 4 °C for a further 24 h. Sections were washed twice in PB, incubated in a mixture of the appropriate secondary antisera diluted in CTA for 1 h at RT then washed twice in PB and coverslipped with DakoCytomation Fluorescent Mounting Medium. Negative control sections were treated as above, with the omission of the primary antibody.

In double labeling studies, cryostat sections were incubated in a mixture of the primary and subsequently of the secondary antibodies. To control for cross-reactivity of the secondary antibodies and spectral bleed through, double immunofluorescence staining was performed by omitting one of the primary antibodies and examining the sections through both fluorescence channels appropriate for each of the secondary antibodies.

HR1 blocking peptide control experiments were performed on sections of mouse retinae prepared as described above. The peptide immunogen for HR1 was CNENFKKTFKKILHIRS (Alpha Diagnostic International, San Antonio, TX, USA), corresponding to a region at the C-terminus of the cytoplasmic domain of rat HR1.