5,7-DIHYDROXYTRYPTAMINE DESTROYS INDOLEAMINE-ACCUMULATING CELL BODIES IN CARP RETINA

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Effects of 5,7-dihydroxytryptamine (5,7-DHT) on indoleamine-accumulating (IA-) cells were investigated on the retinas (cryo-sectioned or flat-mounted) of adult carp (*Cyprinus carpio*), by means of a histofluorescence technique. The eyes, pretreated with a single intravitreal injection of 5,7-DHT (15 μ g), were removed at various testing periods (4 hr to 50 d) with or without an intravitreal injection of 5,6-dihydroxytryptamine (5,6-DHT; one or 5 μ g) alone, or with the same injection of noradrenaline (NA; 5 μ g) plus 5,6-DHT (5 μ g) 2 hr prior to enucleation. In cryo-sections of the retinas loaded with 5,6-DHT (5 μ g), the indoleamine was found to be taken up not only by IA-cells but also by other cells located in the amacrine cell layer. The latter class of cells was more weakly fluorescent and smaller in size than IA-cells. Two days after pretreatment with 5,7-DHT, IA-cells already became invisible, while the accumulative ability of smaller cells for 5,6-DHT remained. Subsequently, IA-cells were not seen again with 5,6-DHT in flat-mounts up to 50 d (the longest testing period in the present experiment) after pretreatment with 5,7-DHT. The results obtained reveal that intravitreally given 5,7-DHT (15 μ g) destroyed quasipermanently the somata of retinal IA-cells in the adult carp.

Ehinger and Floren (4, 5) demonstrated histochemically in radial sections of the retinas of various species of vertebrates (including goldfish) that an intravitreal injection of 5,7-dihydroxytryptamine (5,7-DHT) destroys terminals of indoleamine-accumulating (IA-) cells, retaining the somata almost intact. Following their technique for the carp retina, we found in flat-mounts that the somata of IA-cells also become invisible (8). Since the discrepancy in findings by the earlier workers and by us was assumed to be due to differences in the preparations used, we stated that pale somata of yellowish IA-cells might be observed together with shiny somata of greenish DA-cells in cryo-sections of retinal samples pretreated with 5,7-DHT (15 μ g) one week earlier and subsequently with noradrenaline (NA; 5 μ g) plus 5,6-dihydroxytryptamine (5,6-DHT; 5 μ g) 2 hr prior to enucleation. However, our recent experiments with flat-mounted and cryosectioned preparations have revealed that the somata of IA-cells cannot be seen even just a few days after a single pretreatment with 5,7-DHT, and do not reappear up to 50 days afterwards. The results indicate that IA-cells in the fish retina are much more susceptible to 5,7-DHT than the description by Ehinger and Floren (4, 5) on retinal IA-cells of various species of vertebrates. The present paper is supplemental to our previous paper (8) and

demonstrates that 5,7-DHT destroys quasi-permanently retinal IA-cells in the adult carp.

MATERIALS AND METHODS

A histochemical study was carried out on the retinas of carp (*Cyprinus carpio*), ranging from 30 to 35 cm in body length (tip-to-tip). The size of the eye-ball examined was approximately 13.0 mm in diameter and 1.3 g in weight on the average ($n=24$). The fish were dark-adapted for 1 h before enucleation to facilitate the separation of the retina from the pigment epithelium. The isolated retinas were immersed overnight at 4°C in a fixative, FGS solution, consisting of 4% paraformaldehyde, 0.5% glutaraldehyde and 35% sucrose in 0.1 M phosphate buffer at pH 7.4 (6–8).

After fixation, the retinas were flat-mounted on a glass plate with the vitreal side facing up, or they were cryo-sectioned. For flat-mounts, after the vitreous body was gently removed from the retinal tissue, they were dried over Drierite in a desiccator for one day, sealed with Entellan and examined under a fluorescence microscope (Nikon EF). The cryo-sections were dealt with in the same way.

The present study consisted of four series of experiments, which will be described in such an order as to follow our developed strategies. In series I, one or 5 μg 5,6-DHT was injected into the eyes 2 hr prior to enucleation. For series II, the eyes were pretreated with a single injection of 15 μg 5,7-DHT one week before and injected with one or 5 μg 5,6-DHT 2 hr before eye removal. The retinas fixed in the FGS solution were split up into two halves; one of them was flat-mounted and the other was cryo-sectioned radially or tangentially to 15 μm thickness. In series III, 15 μg 5,7-DHT was injected into the eyes, which were removed at various testing periods (4, 8, 12, 24 and 48 hr). For series IV, 15 μg 5,7-DHT was injected into the eyes, which were removed at longer testing periods (2, 7, 14, 28 and 50 d) in combination with an intravitreal injection of 5 μg NA plus 5 μg 5,6-DHT 2 hr prior to enucleation. The retinas were examined as flat-mounts in series III and IV.

TABLE I. *Experimental protocols*

Series	Drug	Quantity	Testing Period	Number of Retinas
I	5,6-DHT	1 or 5 μg	2 hr	8
II	5,7-DHT	15 μg	7 d	8
	5,6-DHT	1 or 5 μg	2 hr	
III	5,7-DHT	15 μg	4–48 hr	12
IV	5,7-DHT	15 μg	2–50 d	17
	NA plus 5,6-DHT	5 μg each	2 hr	

5,6-DHT, 5,6-dihydroxytryptamine creatinine sulfate (Sigma); 5,7-DHT, 5,7-dihydroxytryptamine creatinine sulfate (Sigma); NA, noradrenaline bitartrate (Wako). The above quantity of monoamines was dissolved in 10 μl fish Ringer's solution with 10 μg ascorbic acid as antioxidant. In some cases, pargyline HCl (15 μg ; Sigma) was added to 5,7-DHT, but the results were the same with or without this monoamine-oxidase inhibitor.

The experimental protocols are listed in Table I. Further description of the procedures will be given in appropriate sections in Results, because it would be helpful for understanding of the results obtained.

RESULTS

In series I, when a dose of 5 μ g 5,6-DHT was intravitreally injected into the eyes 2 hr prior to enucleation, it was found in cryo-sections that many cells took up

FIG. 1. Fluorescence photomicrographs of tangential sections (15 μ m thickness) through the amacrine cell layer in different retinal preparations, showing greenish DA-cells (marked by "g"), and larger and smaller yellowish cells (marked by "ly" and "sy" respectively). A: From the eye injected with 5 μ g 5,6-DHT 2 hr before enucleation. B: From the eye injected with one μ g 5,6-DHT 2 hr before. C: From the eye pretreated with 15 μ g 5,7-DHT one week earlier and injected with 5 μ g 5,6-DHT 2 hr before enucleation. D: From the eye pretreated with 15 μ g 5,7-DHT one week earlier and injected with one μ g 5,6-DHT 2 hr before. Magnification: A–C $\times$ 560

FIG. 2. Fluorescence photomicrographs showing changes in visible cells at various testing periods after a single intravitreal injection of 5,7-DHT (15 μ g). All pictures were taken from corresponding intermediate regions of different retinas. A: From the eye removed 4 hr after the injection. There are many visible cells (white-yellowish in color) of various sizes. Among them, however, the largest-sized cells are endogenously DA-containing cells (greenish), one of which is marked by "g". B: From the eye removed 2 days after pretreatment with 5,7-DHT. Pale DA-cells are seen, while others are almost undetectable. C: From the eye removed 2 hr after injection of NA (5 μ g) plus 5,6-DHT (5 μ g) and 2 d after pretreatment with 5,7-DHT. Greenish fluorescence of DA-cells is enhanced by uptake of NA, while IA-cells are not visible. D: From the eye (one of 4 samples) removed 2 hr after NA plus 5,6-DHT and 50 d after 5,7-DHT. IA-cells do not become fluorescent. A–D $\times$ 400

the indoleamine and even endogenously dopamine-containing (DA-) cells became slightly yellowish (Fig. 1A). The number of yellowish cells other than DA-cells seen in cryo-sections certainly exceeded that of yellowish cells which were counted in flat-mounts made of the other half of the same retinas. In cryo-sections, yellowish cells other than essential DA-cells (Fig. 1A, "g") could be classified into two subsets; one was heavily yellowish and larger in size (Fig. 1A, "ly") and the other was weakly yellowish and smaller (Fig. 1A, "sy"). When the population ratio of smaller cells to larger cells was estimated from cell counts in photomicrographs (0.012 mm^2) containing one of the larger cells, it was 1 : 3.5 on the average ($n=25$). Based on the cell size, indoleamine-accumulating (IA-) cells visible in flat-mounts were assumed to correspond to the larger yellowish cells in cryo-sections. Subsequently, one μg of 5,6-DHT was given to the eyes 2 hr before enucleation. In cryo-sections of these retinas, only the subset of larger yellowish cells was observed with endogenous DA-cells (Fig. 1B); the number of the former cell was comparable to that of IA-cells in flat-mounts.

To further confirm the above assumption, the eyes were pretreated with an intravitreal injection of 5,7-DHT ($15 \mu\text{g}$) in series II. One week later, one or $5 \mu\text{g}$ of 5,6-DHT was injected into the same eyes 2 hr before enucleation. In cryo-sections of the retinas injected with one μg 5,6-DHT, no yellowish cells were visible (Fig. 1D). In the retinas with $5 \mu\text{g}$ 5,6-DHT, on the other hand, only small and weakly yellowish cells (Fig. 1C, "sy") were seen with DA-cells (Fig. 1C, "g"). Therefore, these residual small cells had not been affected by 5,7-DHT given one week earlier. The findings indicate that the small and weakly yellowish cells do not belong to the class of IA-cells, and that the dose of $5 \mu\text{g}$ 5,6-DHT is already excessive for identification of IA-cells in cryo-sections, and thus the indoleamine is taken up by other amacrine cells which are smaller in size than IA-cells. Since the IA-cells took up 5,7-DHT much more than the smaller cells did, the former would be predominantly affected by the neurotoxin.

The above results suggest that IA-cells in the carp retinas are highly sensitive to 5,7-DHT. However, we cannot judge which could be the case that the uptake mechanism of IA-cells for 5,6-DHT is prevented or that IA-cells are really destroyed by 5,7-DHT. Consequently, we planned to explore detailed courses of deterioration of IA-cells in series III and IV. A single intravitreal injection of 5,7-DHT ($15 \mu\text{g}$) was given to the eyes, which were enucleated with or without a prior injection of 5,6-DHT ($5 \mu\text{g}$) plus NA ($5 \mu\text{g}$) at various testing intervals. In flat-mounts of retinas, made within a period from 4 to 24 hr after a single injection of 5,7-DHT, many cells of various sizes were seen emitting a white-yellowish fluorescence, resulting from their uptake of 5,7-DHT (Fig. 2A). Endogenous DA-cells were seen weakly, emitting proper greenish fluorescence (Fig. 2A, "g"). When the eyes were enucleated 2 days after the injection of 5,7-DHT, white-yellowish fluorescence of individual cells became faint; DA-fluorescence was also pale (Fig. 2B). At this stage, if a mixture of NA ($5 \mu\text{g}$) and 5,6-DHT ($5 \mu\text{g}$) was injected into the eyes 2 hr prior to enucleation, greenish DA-cells were easily seen while IA-cells were already undetectable (Fig. 2C). Fluorescence micrographic pictures at this stage were similar to those observed in preparations which were made one week after injection of 5,7-DHT, as previously shown (8). Yellowish IA-cells did not reappear for up to 50 d, which was the longest testing period in the present experiment (Fig.

2D). The results strongly indicate that IA-cells were destroyed by a single intravitreal injection of 5,7-DHT (15 μ g) in the adult carp.

DISCUSSION

The present results confirmed the previous findings (8) that IA-cells become invisible one week after intravitreal injection of 5,7-DHT (15 μ g), and demonstrated for the first time that the somata of IA-cells are quasipermanently destroyed by the neurotoxin in the retina of adult carp (30–35 cm in body length). Furthermore, in cryo-sectioned preparations (15 μ m thickness), uptake of 5,6-DHT by cells other than IA-cells located in the amacrine cell layer could be visualized when an excessive dose of 5,6-DHT was intravitreally given. Those cells were smaller in size than IA-cells and accumulated less indoleamines than did IA-cells, and thus they were neither detectable in flat-mounts nor destroyed by previously injected 5,7-DHT.

When the retinal flat-mounts were examined 4–24 hr after an intravitreal injection of 5,7-DHT, many fluorescent cells (white-yellowish in color) that had taken up the neurotoxin were observed in addition to endogenous DA-cells (greenish). The former group of visible cells may include IA-cells and smaller cells which were detected in cryo-sections of the retinas preloaded with 5,6-DHT. We have failed to classify these smaller cells which possibly take up the indoleamines non-specifically.

Ehinger and Floren (4, 5) demonstrated in radial sections of the retinas of various vertebrates including goldfish that intravitreal injection of 5,7-DHT destroys only the processes and terminals of IA-cells, preserving the somata, which retain the accumulative ability for 5,6-DHT almost intact. Only a few reports are available concerning the destruction of neuronal somata by 5,7-DHT in the central nervous system (CNS); 5,7-DHT, injected into the lateral ventricle of the rat brain, destroyed mainly axons and axon-terminals of both serotonin and noradrenaline neurons (2). Baumgarten and Björklund (1) stated that the higher dose of 5,7-DHT produces lesions in both serotonin and noradrenaline axons which lie closer to the cell bodies, resulting in a higher incidence of retrograde cell loss and impaired regeneration. In the carp retina, IA-cells located in the amacrine cell layer are close to the vitreous body (20–30 μ m apart from the vitreal surface of the retina), into which 5,7-DHT is injected. A large dose (15 μ g) of the neurotoxin would destroy the IA-cell bodies within a few days following the intravitreal injection; this was demonstrated in the present study. Retinal IA-cells do not contain indoleamines, or at least they have not been visualized without preloading of 5,6-DHT. Such a class of cells might be more susceptible to 5,7-DHT than serotonin and noradrenaline neurons are in the CNS. The effects of 5,7-DHT on IA-cells are apparently dose-dependent.

Another neurotoxin, 6-hydroxydopamine (6-OHDA) destroyed selectively retinal dopaminergic (DA-) cell bodies in the adult carp (8). However, the destruction with 6-OHDA was not homogenous over all the retinal area; some DA-cells remained as a cluster in some areas, especially in the central and intermediate regions while no DA-cells were found in other areas particularly in the peripheral region (8). The destructive effect of 6-OHDA seems also to be quasipermanent (Negishi, personal communication), and detailed studies are in progress on morphological changes of residual DA-cells for a long term. The degenerating course

of the retinal cells with 5,7-DHT as well as with 6-OHDA appears to be similar to that of noradrenaline neurons with intraventricular 6-OHDA in the rat locus coeruleus (3). The rapid destruction of retinal IA-cell bodies with 5,7-DHT, observed in the present study, might be mainly due to the neurotoxic effect of 5,7-DHT (2) directly taken up by the somata of IA-cells.

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