which was cut to permit outflow of perfusate. Arteries were perfused with a Harvard peristaltic pump at constant flow rate (2-8 ml/min); perfusion pressure was monitored (Statham P23Dc pressure transducer) and recorded on paper (Servogor S). Drugs were delivered by bolus injection (10–100 µl) into an injection cuff in the perfusion line. Injection of adrenaline and noradrenaline (10^{-5}–10^{-8} g) produced a biphasic response of an initial vasoconstriction followed by a longer period of vasodilation (Figure a); isoprenaline produced only vasodilation. The β-blocker, propranolol hydrochloride potentiated the constrictor phase and diminished the dilator phase (Figure b). Isoprenaline given after propranolol had no constrictor effect but produced a diminished dilator response. Phentolamine mesylate diminished the enhanced constrictor response following propranolol. All arteries examined were qualitatively similar in response. Spontaneous activity and the frequent increase in tone in these preparations has to date precluded quantifying the response to catecholamines.

These results demonstrate that catecholamines can mediate inhibitory effects on the aorta and afferent branchial arteries supplying blood to the gills of the elasmobranch fish *Squalus acanthias*.

Zusammenfassung. Nachweis, dass Catecholamine hemmende Wirkungen auf die Aorta und die afferenten branchialen Arterien, welche die Kiemen des Knorpelfisches *Squalus acanthias* mit Blut versorgen, vermitteln.

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Interaction Between 6-Hydroxydopamine and Rhodopsin in vivo in the Rat Retina

Since dopamine has been suggested to be an inhibitory neurotransmitter in the retina[1,2] and since 6-hydroxydopamine (6-OH-DA) has been widely used recently to study the functions of adrenergic neurons[3], it was considered appropriate to use 6-OH-DA to test the functional effects of dopaminergic retinal neurons. The mechanism of action of 6-OH-DA in inducing the degeneration of adrenergic nerve terminals is still uncertain. WAGNER[4] reported that 6-OH-DA uncouples phosphorylation from electron transport similar to the classical uncoupler, 2,4-dinitrophenol, and suggested that this property of 6-OH-DA may be the first step in initiating the degeneration. 6-OH-DA is readily auto-oxidized resulting in the

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Effects of drugs on retinal sensitivities and rhodopsin contents

Compound	Dose (µmole/eye)	Sensitivity (N.D.No.)	*N*	Rhodopsin[a] (%)	*N*
Saline	—	4.75 ± 0.08	18	100.0 ± 7.1	16
6-Hydroxydopamine · HBr	0.26	4.76 ± 0.12	12	99.5 ± 15.5	4
	0.51	2.28 ± 0.32	10	43.3 ± 13.3	4
	1.0[b]	0.43 ± 0.16	19	13.8 ± 3.8	4
6-Hydroxydopamine · HBr	1.0				
+ $Na_2S_2O_5$	0.26	0.55 ± 0.36	6	27.6 ± 8.5	6
	0.51	2.65 ± 0.41	3	72.5 ± 9.1	4
	1.0[b]	3.45 ± 0.32	6	85.4 ± 8.2	6
$Na_2S_2O_5$	0.26	4.47 ± 0.15	4	90.9 ± 1.8	3
	0.51	3.88 ± 0.11	4	101.2 ± 4.5	3
	1.0[b]	3.24 ± 0.16	6	100.3 ± 11.0	4
2,4-Dinitrophenol[c]	2.0	3.90 ± 0.06	8	—	
	4.0	3.85 ± 0.04	6	—	
0.5 *M Tris*-HCl buffer, pH 9.5	—	4.12 ± 0.06	6	100.0 ± 4.7	6
H_2O_2	4.0	3.47 ± 0.14	11	—	
6-Aminodopamine · 2HCl	0.26	2.00 ± 0.08	5	51.0 ± 2.6	5
	0.51	1.06 ± 0.12	5	35.8 ± 7.1	5
	1.0	0.11 ± 0.06	8	14.3 ± 2.1	7
p-Quinone · HBr of 6-hydroxydopamine	1.0	0.75 ± 0.32	6	27.5 ± 2.9	6
p-Chloromercuribenzoate, Na[c]	1.0	0.18 ± 0.11	5	24.2 ± 7.8	5

Light-adapted rats were anesthetized with ether, injected intravitreously with 10 µl of solvents or drug solutions, and subsequently dark adapted for 4–6 h before the sensitivity and rhodopsin content were measured as described [15]. Sensitivity was expressed as the Kodak neutral density filter number (N.D.No.) placed in the stimulus light path. Rhodopsin content of dark-adapted, saline-injected control served as 100%.
[a] Mean ± S.E. [b] Data from PONG and GRAHAM [15]. [c] In 0.5 *M Tris*-HCl buffer, pH 9.5.

generation of a simple *p*-quinone[5–7] (2-[β-aminoethyl]-5-hydroxy-*p*-benzoquinone) and H_2O_2[8,9]. SANER and THOENEN[10] suggested that the covalent binding of the *p*-quinone with nucleophilic groups of biological macromolecules would lead to a functional impairment of the structure involved. HEIKKILA and COHEN[8,9,11] proposed that the cytotoxicity of H_2O_2 may be responsible for the degeneration. Recently, 6-aminodopamine (6-ADA) has been demonstrated to be as effective as 6-OH-DA in chemical sympathectomy[12–14]. We previously reported[15] that 6-OH-DA, when administered intravitreously to light-adapted rats, unexpectedly prevented the recovery of retinal sensitivity during subsequent dark adaptation, probably through its interaction with photochemical mechanisms. In order to test the hypotheses relating to the mode of action of 6-OH-DA, the interaction between 6-OH-DA and rhodopsin in vivo in the rat retina was further examined to include 2,4-dinitrophenol, H_2O_2, *p*-quinone, 6-ADA, $Na_2S_2O_5$ and *p*-chloromercuribenzoate.

All experiments were made on mature male light-adapted albino rats (280–350 g). The retinal sensitivity was determined as the minimum light stimulus necessary to evoke a response with a potential of 10 to 20 μV in the b-wave of the ERG[16]. Rhodopsin content was measured by the decrease in absorption at 500 nm upon total bleaching of a digitonin extract of retinal tissue[16]. The animal treatment, measurements of retinal sensitivity and rhodopsin content were essentially as described previously[15]. For the examination of chronic effects, some 6-OH-DA injected rats were allowed to recover and the sensitivities were similarly measured after 1 to 4 weeks. Some eyeballs injected with 1 μmole of 6-OH-DA were removed and histological sections were prepared essentially as described by RICHARDSON et al.[17] for examination of possible morphological alterations of retina at the light microscopic level.

The results are summarized in the Table. 6-OH-DA decreased both the retinal sensitivity and rhodopsin content and the effects were highly dose-dependent. The

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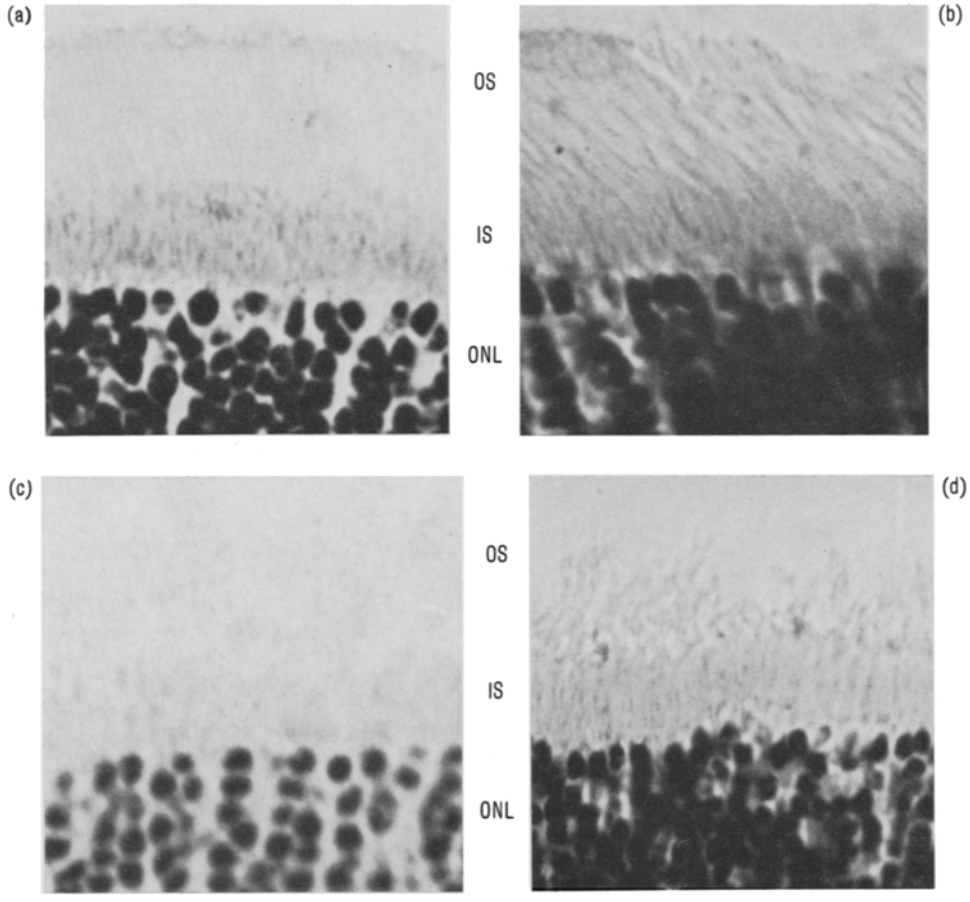

Photomicrographs of methylene blue-stained sections (4 μm, × 760) of rat photoreceptors after intravitreal injection of saline (a); and of 6-hydroxydopamine (1 μmole/eye/10 μl), (b); 2 (c); and 4 (d) days after injection. The sections were cut perpendicular to the retinal surface and prepared essentially as described by RICHARDSON et al.[17]. OS, outer segments; IS, inner segments; ONL, outer nuclear layer.

effect was long-lasting, since, after the injection of 6-OH-DA for 1 to 4 weeks, the retinal sensitivity not only did not recover but decreased even more (data not shown). $Na_2S_2O_5$, an antioxidant, prevented the 6-OH-DA-induced effect and the preventive effect was also dose-dependent and quantitative. $Na_2S_2O_5$ itself had no effect on rhodopsin content and decreased the sensitivity only slightly. Since higher doses (up to 4 μmoles) of 2,4-dinitrophenol and H_2O_2 decreased the sensitivity only slightly, it appears neither uncoupled phosphorylation nor the cytotoxicity of H_2O_2 seem to be directly responsible for the effect of 6-OH-DA. On the other hand, 6-ADA and the *p*-quinone appear to be as effective as 6-OH-DA.

It has been long speculated that a conformation change in the photoreceptor protein (opsin) resulting from light stimulation exposes new active groups such as sulfhydryl[18], amino[19] and proton-binding groups[20]. It is thought that the regeneration of rhodopsin involves the covalent binding of 11-*cis* retinal to the ε-amino group of a lysine residue in the opsin[21,22] or through some form of a substituted aldimine linkage to the lysine amino and a cysteine sulfhydryl group[19]. *p*-Quinone, the primary and principal oxidation product of 6-OH-DA[5-7] could undergo covalent binding with these exposed free sulfhydryl and/or amino groups of opsin in the light-adapted retina, and thus prevent the regeneration of rhodopsin and concomitantly the recovery of retinal sensitivity. Interestingly, *p*-chloromercuribenzoate, a sulfhydryl-blocking agent, was similar to 6-OH-DA in its effect on retinal sensitivity and rhodopsin content.

The interaction between 6-OH-DA and rhodopsin was further supported by histological findings. In all the saline-injected controls (Figure a), the outer and inner segments of photoreceptors were clearly seen. In the 6-OH-DA (1 μmole) treated retina, the outer segments were damaged severely (Figure d) and partially (Figure c) 4 and 2 days, respectively, after injection. 1 day after injection, only part of the outer segments can be seen to be disorganized (Figure b), although functionally the sensitivity was already diminished. The rest of the retina seemed not to be affected.

Thus, the intravitreal administration of 6-OH-DA to light-adapted rats was found to prevent the recovery of retinal sensitivity and rhodopsin during the subsequent dark adaptation probably through the covalent binding of its oxidation product to the nucleophilic groups of opsin. The effect was dose-dependent, long-lasting, blocked by antioxidants, and non-specific to catecholaminergic neurons. Therefore, 6-OH-DA at doses comparable to that employed in this study should not be used to investigate the function of retinal dopamine. The interaction of 6-OH-DA with rhodopsin should be considered in the interpretation of results obtained even at lower doses.

Résumé. Nous avons observé que l'injection de la 6-hydroxydopamine dans l'humeur vitrée des rats adaptés à la lumière bloquait le rétablissement de la sensibilité rétinale pendant l'adaptation à l'obscurité subséquente au traitement. Il est possible que cette observation soit une conséquence de la réaction du produit de l'oxydation de la 6-hydroxydopamine avec les groupes nucléophiliques de la rhodopsine.

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Tolerance Development to the Antinociceptive Actions of Morphine, Amphetamine, Physostigmine and 2-Aminoindane in the Mouse

There have been many attempts to explain the actions of the narcotic analgesics in terms of selective interference with central chemical transmission[1,2]. However, recently several authors who had previously suggested that morphine selectively interferes with one transmitter system have advanced experimental work suggesting a far less specific mechanism of action[3,4]. This point has been highlighted by KUHAR, PERT and SNYDER[5] who were unable to demonstrate any obvious correlation between the distribution of opiate receptors in monkey brain with that of known neurotransmitters, and argued against an exclusive interference with the activity of cholinergic, serotoninergic or noradrenergic neurones.

In addition to the narcotic analgesics, a large number of other compounds possess antinociceptive activity including sympathomimetics (e.g. amphetamine) and cholinomimetics (e.g. physostigmine and oxotremorine). Our interest has been in trying to determine whether there is any correlation between the characteristics of narcotic analgesic activity and those of other agents which might indicate some similarity in mechanism of action.

Whilst the antagonism of the actions of morphine and its pharmacological analogues by the narcotic antagonists separates them from other antinociceptive agents indicating an action at a specific central receptor, there is evidence which connects the antinociceptive action of morphine with that of the sympathomimetics.

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