de l’œil, J. Physiol. Exp. Pathol. 4: 176, 1824.
7. Agaki, K., Nishimura, K., and Yamamoto, A.: Supplementary studies on the consensual ophthalmometric reaction. I. The influence of the changes of the intraocular pressure on one eye on that of the fellow eye and the blood pressure. II. The relation between the consensual ophthalmometric reaction and the cervical sympathetic or trigeminal nerve, Ganka-Kiyo 6: 180, 1955 (Ophthalmic Literature, Vol. 9, No. 898).

Analysis of clonidine-induced mydriasis.

MICHAEL C. KOSS AND LUKE C. SAN.

In addition to its centrally mediated hypotensive action, clonidine causes a decrease in intraocular pressure associated with a long-lasting mydriasis. The present study was conducted to determine to what extent this drug-induced pupillary dilation is of central or peripheral origin. Pupil size was observed in cats anesthetized with pentobarbital. Clonidine (1 to 100 µg per kilogram, intravenously) resulted in a dose-dependent increase in pupillary diameter in intact as well as sympathectomized preparations. These same doses of clonidine produce no effect on the eserinizd, parasympathectomized iris. Epinephrine administration (0.1 to 30 µg, intra-arterially) produced an equivalent pupillary dilation in all preparations. In addition, clonidine caused a dramatic decrease in postganglionic ciliary nerve activity and both the decreased nerve activity and pupillary dilation were reversed by intravenous administration of yohimbine hydrochloride. These results suggest that the inhibition of parasympathetic tone by clonidine may involve a central adrenergic inhibitory mechanism.

The hypotensive drug clonidine lowers blood pressure by acting directly on the central nervous system. Recent investigations suggest that clonidine activates an alpha-adrenergic mechanism located in the medulla oblongata or hypothalamus. In conjunction with other long-lasting central nervous system (CNS) actions, such as sedation and inhibition of respiration, clonidine also exerts a transient sympathomimetic action on a variety of peripheral sympathetic systems.

In addition to its hypotensive action, clonidine has been shown to produce a therapeutically useful decrease in intraocular pressure associated with a long-lasting pupillary dilation. The aim of the present study was to quantitatively describe the mydriatic action of clonidine in the cat with regard to the site and mechanism of action involved.

Methods. Adult cats of either sex were anesthetized with pentobarbital (36 mg per kilogram, intraperitoneally). Following cannulation of the trachea, femoral artery, and vein, the animals were placed in a Kopf stereotaxic instrument. Blood pressure was recorded from the femoral artery by means of a Statham P23Dd pressure transducer and displayed on a Grass polygraph (Model 7B). Pupillary responses were measured directly with a millimeter ruler at the point of greatest horizontal diameter and photographed either directly or with the aid of a camera attached to an operation microscope (Olympus Model MTX). All observations were made under the same general ambient lighting conditions.

In some preparations the cervical sympathetic nerve trunk was sectioned preganglionically at the midcervical level and in others the medial and lateral short ciliary branches of the oculomotor nerve were cut following a lateral intraorbital approach. In these latter preparations, the subse- quently dilated pupils were constricted by topical application of 1 to 2 drops of a 1 per cent solution of phystostigmine salicylate. The direct effect of epinephrine was observed in both types of preparations following injection (0.2 ml) into the lingual artery.

In one series of experiments, bipolar platinum electrodes were placed beneath the lateral short ciliary nerve and the effect of clonidine on the activity in this postganglionic nerve was observed. The nerve was covered with warm mineral oil and the electrical activity amplified by a differential amplifier (Tektronix Model 26A2). These amplified potentials were displayed on a dual-beam storage oscilloscope (Tektronix Model D13) and were integrated (Grass Model 7P10B) for display on a polygraph (Grass Model 7B). A Hewlett Packard (Model 3960) F-M tape recorder was utilized for storage.

Drugs were dissolved in physiological saline and the doses are expressed in terms of their salts. Drugs employed were: clonidine hydrochloride (Boehringer Ingelheim, Ltd.), yohimbine hydrochloride (Boehringer Ingelheim, Ltd.), and physostigmine salicylate.
hydrochloride (Aldrich Chemical Co., Inc.), hexamethonium chloride (Nutritional Biochemicals Corp.), atropine sulfate (Nutritional Biochemicals Corp.), and epinephrine hydrochloride (Parke, Davis & Co.).

Results.

Clonidine on neurally intact iris. Intravenous administration of clonidine (1 to 100 μg per kilogram) resulted in a dose-dependent pupillary dilatation when both the sympathetic and parasympathetic innervation of the iris was intact. The onset of this clonidine-induced mydriasis was seen at approximately 10 to 30 seconds following the injection, reached its maximum within the first minute, and thereafter remained constant. The pupillary dilation in response to the smaller dosages was unchanged for at least 2 hours in four animals observed for this length of time and in six animals the larger dosages maintained their full mydriatic effect for at least 6 hours. In no case was any recovery observed in these acute preparations.

Subsequent intravenous administration of yohimbine (0.5 to 1.0 mg, per kilogram) effectively reversed the clonidine mydriasis, having a latency of onset of 20 to 60 seconds and reaching its maximal effect in 2 to 5 minutes. In all cases, intravenous administration of atropine resulted in the expected mydriasis due to its peripheral muscarinic blocking actions. The clonidine dose-response relationships as well as the antagonistic effect of yohimbine are summarized in Fig. 1.

Sympathetic vs. parasympathetic innervation. In six preparations the sympathetic innervation to the iris was cut on one side and the parasympathetic nerves to the iris sectioned on the other. Intravenous administration of clonidine (1 to 100 μg per kilogram) resulted in a pupillary dilatation of only that eye with the parasympathetic innervation intact and, as before, this mydriatic effect of clonidine was reversed by yohimbine (Fig. 2). Clonidine had no observable effect on any of the parasympathectomized preparations. As seen in Fig. 2, these preparations responded in the expected manner to intravenously administered hexamethonium (Cc) as well as to atropine. Fig. 3 summarizes this selective action of clonidine in these animals.

Ciliary nerve recordings. The effects of clonidine and subsequent administration of yohimbine on the activity of the lateral ciliary nerve were observed in four experiments. Parasympathetic nerve activity was recorded from the left and the effect of these agents on pupil size observed on the right. A single dose of clonidine (30 μg
Fig. 2. This series of photographs illustrates the comparative effect of drugs administered intravenously on the parasympathectomized (left) vs. the sympathectomized (right) cat iris. Note that clonidine caused mydriasis only in the eye with parasympathetic innervation intact. Following reversal of clonidine’s effect by yohimbine, hexamethonium (Co) and atropine exert their expected actions in this preparation.

Fig. 3. Composite representation of the cumulative dose-response to clonidine (1 to 100 μg per kilogram, intravenously) on the pupils in six cats. On one side the parasympathetic nerve to the iris was sectioned and the pupil constricted by topical physostigmine (dashed line). On the other side the sympathetic nerve was sectioned (solid line). Note that clonidine acts only if the parasympathetic tone is intact. Vertical bars are ± S.E.M.

Discussion. The results of this investigation demonstrate that intravenous administration of clonidine produces a long-lasting, dose-dependent pupillary dilation in the anesthetized cat. Although clonidine-induced mydriasis has been previously observed by other investigators in the cat as well as in the rat and in man, no previous studies regarding the site or mechanism of action have been undertaken. Most investigators have assumed a direct action of clonidine on the iris as this agent has been shown to be a peripheral alpha-adrenergic agonist on a variety of other sympathetic organs. As clonidine is devoid of nicotinic ganglionic blocking actions, the present experiments indicate that the pupillary dilation observed is a result of a CNS inhibition of the parasympathetic tone to the iris. This effect was demonstrated in experiments utilizing selective nerve section and more directly by the recording of ciliary nerve activity. The lack of any observable peripheral effect on the alpha-adrenergic receptors in the iris was surprising. However, the experiments demonstrating that the parasympathectomized, eserinized iris responds to epinephrine in the same way as the intact iris indicate that there is little if any antagonism of a direct adrenergic action by physostigmine in these preparations.
Fig. 4. Effect of sequential intravenous injections of clonidine, yohimbine, atropine, and hexamethonium (Co) on the neurally intact iris of right eye (photographs) and the integrated nerve activity in lateral short ciliary nerve (SCN) of the left eye. In these experiments the sympathetic nerve supply to the left eye was sectioned. The solid lines leading to the photographs indicate points at which photographs were taken. Note that clonidine decreases ciliary nerve activity to almost the same degree as seen after ganglionic blockade.

The long duration of the mydriatic effect of clonidine further supports the hypothesis of a central action for this agent. Although a direct peripheral alpha-adrenergic activation by clonidine has been demonstrated in many sympathetic structures, the effect is quite transient.\textsuperscript{1-3, 5} For example, the time required for 50 per cent relaxation of clonidine's contraction of the nictitating membrane ranged from 32 minutes at a dose of 10 µg per kilogram to 65 minutes at the 100 µg per kilogram level.\textsuperscript{3} As in the present study, a similar long duration of action for clonidine on the pupil has been observed in both the rat\textsuperscript{9} and man.\textsuperscript{8} The decrease in intraocular pressure in man has also been reported to have a comparable long duration.\textsuperscript{6-8}

One characteristic of clonidine is that its central inhibitory action is competitively antagonized by the alpha-adrenergic blocking agents yohimbine and piperoxane. It is well established that pretreatment with these agents not only reduces the CNS inhibitory actions of clonidine (as measured by blood pressure, heart rate, and sympathetic nerve activity) but also reverses the sympathoinhibitory action if given after clonidine.\textsuperscript{2} Other investigators have demonstrated a blockade of clonidine's respiratory depression by these two agents.\textsuperscript{1}

The results of the present investigation are consistent with the above studies in that yohimbine was found to consistently reverse the mydriasis produced by clonidine. These observations suggest that clonidine inhibits central reactivity in this parasympathetic system in a manner analogous to its action on a variety of sympathetic systems, and that a central adrenergic inhibitory mechanism may be involved.

The authors gratefully acknowledge the technical assistance of Miss Sarah Ervin during the conduct of these experiments.

From the Department of Pharmacology, University of Oklahoma College of Medicine, Oklahoma City, Okla. This investigation was supported by a Fight For Sight Grant, Fight For Sight, Inc., New York, N. Y. Presented in part at the Southern Section Meeting of the Association for Research in Vision and Ophthalmology, Houston, Texas, Nov. 7, 1975. Submitted for publication.
Actin filaments in apical projections of the primate pigmented epithelial cell. Beth Burns and Alan M. Laties.

A highly-ordered array of filaments is found within the apical processes of retinal pigmented epithelial cells in monkeys and humans. These filaments, approximately 100 A in diameter and 250 A apart, line the cytoplasmic face of the plasma membrane, in parallel with the long axis of the apical processes.

Since these filaments bind rabbit myosin subfragment-1 to form arrowhead complexes, we conclude that they contain actin. Such membrane-bound actin filaments could have any of several different functions: they could stabilize the apical projections and by so doing play a cytoskeletal role, and/or they could take part in the phagocytosis of shed outer segment discs.

Recently, it has become clear that virtually all eukaryotic cells contain actin filaments within their cytoplasm. This view is based not only on biochemical identification of actin extracted from numerous types of cells but also on a powerful technique which allows us to specifically identify actin filaments in situ. This procedure depends upon the specific binding of myosin molecules to actin filaments. When actin-binding parts of myosin molecules are incubated with actin filaments, they attach to form a characteristic arrowhead configuration which is visible with the electron microscope. These arrowhead complexes are considered specific indicators for actin.

Arrowhead complexes may be formed with isolated actin filaments, or in situ in cells which have been glycerinated to make their membranes permeable to the large myosin fragment. Fragments of myosin, either subfragment-1 or heavy meromyosin, are necessary since the intact myosin molecules are insoluble at physiological ionic strengths and would thus not penetrate the cells.

In the eye this technique has already been applied to the study of melanosome motility in the pigment epithelial cells of amphibia and of fish. In mammals, melanosomes are, as well as anyone knows, stationary. However, there remain the motility problems of ingestion and translocation of phagosomes in the retinal pigment epithelial cell. To this end, we have studied the distribution of actin filaments in the pigment epithelial cells of the squirrel monkey, Saimiri sciureus using myosin subfragment-1 binding to identify actin.

Methods and materials.

Subjects. Under deep barbiturate anesthesia (35 mg. Nembutal per kilogram intravenously) the eyes of an adult squirrel monkey (S. sciureus) were enucleated, rapidly dissected in cold standard salt solution (SSS) and then immersed in an appropriate solution as described below. A human retina, from surgical enucleation for orbital tumor, was prepared in the same manner.

Electron microscopy. Retinas were immersion-fixed in 3 per cent glutaraldehyde (Fisher, reagent grade) in SSS consisting of 0.1 M KCl, 5 mM MgCl₂, 6 mM Na-phosphate buffer at pH 7.0, to which 1 mM dithiothreitol (DTT) had been added. After fixation for two to three hours at room temperature, retinas were washed in SSS,