THE REACTION OF THE ISOLATED CAT IRIS TO SEROTONIN[1]

WERNER P. KOELLA AND ULRICH SCHAEPPI

WITH THE TECHNICAL ASSISTANCE OF SANDRA GUSTAFSON AND ANNA BLOCH

Laboratory of Neurophysiology, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

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Intracarotid administration of serotonin (5-HT) to a waking cat induces a slight dilatation of the pupil immediately followed by a marked miosis (Page, 1952, 1954). This effect is restricted to the eye ipsilateral to the side of injection. The contralateral iris usually reacts with a slight dilatation. Furthermore, constriction is obtained only if fairly large doses of serotonin are used, whereas with smaller doses bilateral dilatation ensues in the awake animal. This rather complex pattern, which has been observed a number of times (Koella and Schaeppi, unpublished data), defies a simple explanation on the basis of one locus of action of the drug. In connection with the behavioral and EEG arousal reactions which follow the intracarotid injection of serotonin in the awake animal, the pupillary response could be interpreted in the following preliminary way: The change in size of the pupil in response to this drug is the resultant of at least two competing influences: (1) a direct constrictive effect of 5-HT upon the iris restricted to the side of injection and (2) a shift towards sympathetic preponderance and thus bilateral pupillo-dilating outflow paralleling a generalized central arousal, this latter effect being the result of drug action in the carotid sinus area as well as at intracranial sites (Koella *et al.*, 1960a, b; Koella and Czicman, unpublished data). Furthermore, it is possible that serotonin acts upon the ciliary and the superior cervical ganglion and thus, by alteration of synaptic transmission, introduces two complicating factors. Reid and Rand (1951) reported that "the isolated pupil of the cat in a 5 ml bath of Ringer solution constricts on the addition of 1 or more mg. of vasoconstrictor" (identified as serotonin). Even if one takes into account that Reid's substance may not have been purified to a high degree, the doses used seem to be so large that that author's results cannot be used to support our interpretation as outlined before. Consequently, we initiated a study to investigate systematically the effect of serotonin on the isolated iris.

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METHODS. The experimental work was divided into different parts.

In the first the behavior of the isolated but intact and "physiologically" mounted iris was tested in an apparatus similar to that described by Quilliam (1949). In general, small cats with body weight in the 0.5- to 1.5-kg range were used. The eyes were enucleated under pentobarbital anesthesia and the animals then sacrificed. After removal of the posterior half of the bulbus, the corpus vitreum, the lens, and the cornea, the sclera was cut from the equator to form, together with the ciliary body and the iris, a 6- to 8-pointed star. All dissecting preparations were done in cold Ringer solution saturated with oxygen (NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.02%, $MgCl_2$ 0.01%, $NaHCO_3$ 0.1%, Na_2HPO_4 0.005%). The star-shaped scleral ring with the iris was then attached with needles to a cork or wooden board with a center hole and this board was mounted in the perfusion chamber. Ringer solution at 38° to 39°C and equilibrated with 95% O_2 and 5% CO_2 at a flow rate of 8 to 20 ml per minute was used as bathing fluid. During a particular experiment the flow rate through the chamber was kept constant. The capacity of the chamber was approximately 11 ml. The drugs, diluted in 1 ml Ringer solution, were injected into the inlet tube of the perfusion chamber. It was impossible to establish the actual drug concentrations to which the preparation was exposed, due to dilution during the (parabolic) flow in the inlet tube and also due to dilution in the upstream compartment of the perfusion chamber. A dilution factor of 1 to 10 is probably a very conservative figure. Because of the ill-defined magnitude of the drug concentration, we prefer to have the doses indicated in the RESULTS section understood as relative rather than absolute values.

The measurement of the pupillary size was based on photometric principles. A collimated light beam entered the otherwise light-tight perfusion cell and after passing through the pupil was centered upon a photocell, whose conductance was traced on a G.E. inkwriter or a YSI Model 80 recorder, and thus served as indicator of the area of the pupillary opening. A revolving disk supplied with a series of holes of different diameter could be inserted into the light beam in place of the perfusion chamber and served as a calibrating device. The relation between area of the artificial pupils and the record deflections revealed linearity within about 2%.

In the second part of the experimental work we studied the behavior of the force exerted by the isolated sphincter pupillae and an isolated section of the dilator pupillae. The initial preparatory steps were the same as described above. Once the anterior part of the sclera and the iris were exposed, we cut a concentric ring about 2 mm in width which contained most or all of the circular fibers. This constrictor ring was then suspended between two small glass hooks, one fixed to a micrometer, the other suspended from the force-measuring device. The dilator preparation was obtained by cutting a sector from the lateral or medial aspect of the (two-pointed) iris. This strip was caught between two fine silver clamps and mounted in a manner identical to that used for the constrictor muscle. A Grass FT 03 force-displacement transducer with the lowest spring tension was used to sense "isometric" force. A Grass Polygraph, or a G.E. inkwriter coupled to a suitable preamplifier, served as recording instruments. The muscle preparations were submerged in a bath of 125 ml of Ringer solution in which the fluid was constantly exchanged at a rate of about 10 ml per minute. The temperature was kept at 38° to 39°C. A mixture of O_2 (95%) and CO_2 (5%) served to oxygenate and equilibrate the nutrient and also to agitate the bath. The drugs were injected *via* a 4-inch hypodermic needle to the center of the beaker.

Drugs used: Serotonin creatinine sulfate (Nutritional Biochemicals, Cleveland), acetylcholine chloride (California Corporation for Biochemical Research), *l*-epinephrine bitartrate (Winthrop), *l*-norepinephrine bitartrate (Winthrop), atropine sulfate (Merck), D-lysergic acid diethylamide (LSD, Sandoz). All doses indicated refer to the salt of the drugs.

RESULTS. *The intact isolated iris.* Serotonin (5-HT) acted on the isolated iris as a powerful constrictor. The threshold dose appeared to be in the neighborhood of 0.3 μg (fig. 1A). Doses as little as 6.25 and 12.5 μg often induced complete or almost complete closure of the pupil (fig. 1B). Often the tracings revealed two peaks; this biphasic pattern was usually more apparent with larger doses than with smaller ones (fig. 1C). The second and third application of the same dose of serotonin often produced considerably smaller effects than the first one. This "desensitization" often lasted for as long as 1 hour (fig. 1D). Several of our preparations developed a slowly increasing miosis. Administration of serotonin seemed to favor this process. Both the desensitization and the sustained constriction made it often difficult to produce meaningful dose-response curves (fig. 1E, F).

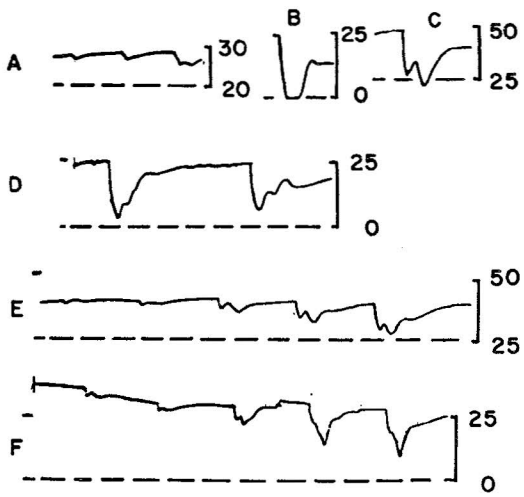

FIG. 1. Effect of serotonin on the isolated intact iris of the cat.

Scale on right of each trace indicates area of pupillary opening in mm². A) 0.39, 0.78 and 1.56 μg of serotonin creatinine-sulfate, respectively, at 15-minute intervals. B) 12.5 μg serotonin. Note complete closure of pupil. Same time scale as in A. C) 3.12 μg serotonin. Note double-peaked response. Same time scale as in A. D) Two injections of 12.5 μg of serotonin at 30-minute interval. Note desensitization effect. E) and F) Dose-response curves: 1.56, 3.12, 6.25, 12.5 and 25 μg at 30-minute intervals.

Acetylcholine (ACh) had also a constrictive effect upon the pupil; the threshold dose, however, was 400 to 1000 times higher than that of 5-HT. An example of the ACh effect is given in figure 2A.

Epinephrine (E) and norepinephrine (NE) produced dilatation of the pupil. The threshold dose of E was 12.5 μg or somewhat lower, whereas the threshold for NE was between 12.5 and 25 μg (fig. 2B).

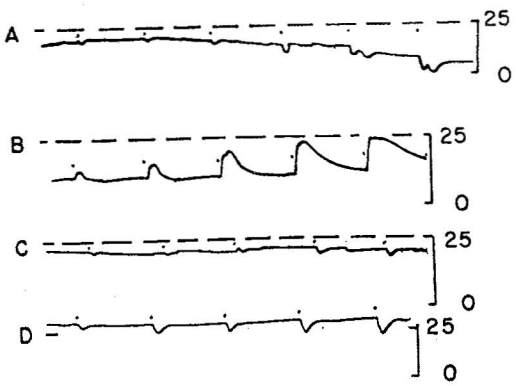

FIG. 2. A) Effect of acetylcholine on isolated iris.
Dose-response curve with 128, 256, 512, 1024, 2048 and 4096 μg at 30-minute intervals. Note slow sustained contraction. B) Effect of epinephrine 12.5, 25, 50, 100, 200 μg at 30-minute intervals. C) Dose-response curve identical to the one shown in figure 1F after 1 mg atropine. D) Dose-response curve identical to the one shown in figure 1E after 1 mg atropine. Note increase in response to small doses of serotonin.

Atropine in doses of from 1 to 16 mg had no direct effect upon the isolated iris, but reduced the response to serotonin (fig. 2C). Occasionally, smaller doses of serotonin were potentiated by atropine (fig. 2D). Atropine always markedly reduced or abolished the effects of acetylcholine.

Large doses of lysergic acid diethylamide (LSD, 100 μg) sometimes inhibited the serotonin response. Because of the self-desensitizing effect of 5-HT, it was somewhat difficult to establish this effect of LSD beyond doubt.

The isolated constrictor pupillae. This preparation reacted to serotonin with a powerful contraction. The threshold dose was about 0.1 μg. This corresponded, assuming homogeneous mixing of the drug in the 125-ml beaker, to a concentration of less than 10^{-9} g per ml. Figure 3A shows a dose-response curve. The maximal tensions produced by this preparation were in the order of 50 mg; 8 to 128 μg of serotonin had to be administered in various preparations in order to induce the maximal degree of contraction. In contrast to the findings on the intact iris, repeated application of the same dose did not reveal desensitization (fig. 3B). With doses ranging from 0.1 to 128 μg, biphasic responses were never observed.

Acetylcholine was considerably less effective. To elicit comparative contractions, the dose ratio of 5-HT to ACh was found to be about 1 to 100. The curves produced by ACh occasionally revealed a biphasic pattern (fig. 3C and C'). The total time of contraction was more prolonged as compared with 5-HT. Occasionally a self-desensitization effect of ACh occurred as evidenced by a progressively decreasing response to repeated administration of identical doses of this drug.

Atropine in doses of up to 100 μg occasionally produced, *per se*, a slight contraction, but did not change the effect of 5-HT (fig. 3D).

LSD in intermediate doses (0.1 to 10 μg) was ineffective in changing the 5-HT effect. Occasionally very small doses of LSD potentiated, and very large doses inhibited the 5-HT effect. Sometimes small amounts of LSD produced a slight contraction of this preparation. This effect never increased with larger doses of LSD.

The isolated dilator pupillae. The sectors taken from the lateral and nasal aspect of the iris, containing radial dilator fibers, reacted to serotonin usually with relaxation followed with larger doses by a prolonged slight contraction. The threshold dose in some of our preparations was as low as 0.03 μg. Figure 4A illustrates the quantitative and qualitative changes in reaction with increasing doses. Figure 4B shows an experiment in which the constrictor and dilator muscle were recorded simultaneously. Norepinephrine induced contraction of the dilator muscle, occasionally preceded by a slight and transient relaxation.

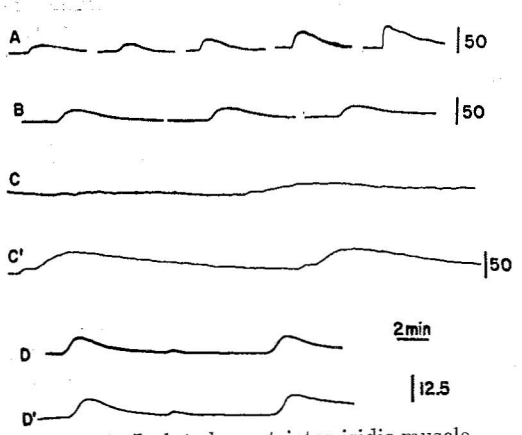

FIG. 3. Isolated constrictor iridis muscle.
Scale at right indicates force in mg. A) Dose-response curve with 0.5, 1.0, 2.0, 4.0 and 8.0 μg of serotonin. B) 4 μg serotonin given three times at 15-minute intervals. Note lack of desensitization. C) Action of acetylcholine 20, 30, 40 and 50 μg. Note slowness of effect. D) Effect of serotonin (1 μg) before and after atropine (10 μg, upper row; 100 μg, lower row).

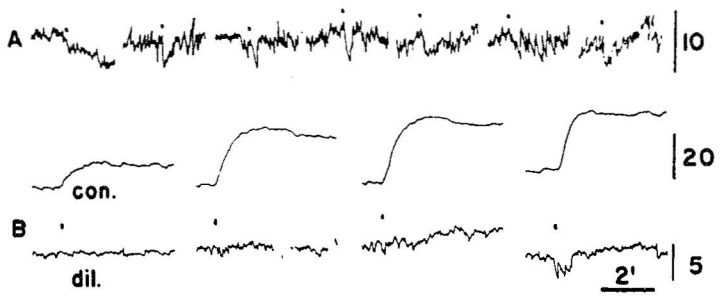

FIG. 4. A) Influence of serotonin on dilator strip of iris.
Scale at right shows force in mg. Doses of 0.015, 0.031, 0.062, 0.125, 0.250, 0.5, and 1 μg were given. Note dilatation (downward) with intermediate doses and biphasic effect with high doses. High sensitivity reveals autonomic activity of preparation. B) Influence of serotonin 0.1, 0.2, 0.4, and 0.8 μg on constrictor (upper) and dilator muscle (lower). Note reciprocal effect. This dilator strip is somewhat less responsive than that shown in A.

DISCUSSION. These experiments, in confirmation of Reid and Rand's findings, demonstrated that serotonin has a direct action on the isolated iris. In our experiments the threshold dose was found to be more than a thousand times smaller than in Reid and Rand's series. The experiments on the isolated constrictor and dilator preparations demonstrated that pupillary constriction in response to serotonin is the result of a dual mechanism: namely, (1) contraction of the (circular) constrictor muscle and (2) relaxation of the (radial) dilator muscle. The isolated muscle never showed a two-peaked response on administration of 5-HT. Close scrutiny revealed that the serotonin effect on the dilator was virtually over at a time when the contraction of the constrictor muscle was still developing. This evidence suggests strongly that the polyphasic behavior observed in the intact iris is in part the manifestation of a mechanical summation of two asynchronous processes. Serotonin induces in the intact iris reciprocal behavior of the two antagonists in an asynchronous time course. Reciprocal behavior in the opposite direction of the iris muscles in response to epinephrine has been described by Hess *et al.* (1950), Hess and Koella (1950), and Koella and Rüegg (1952).

On the intact isolated iris and on the isolated constrictor muscle, acetylcholine was less effective than serotonin. Our isolated constrictor preparations reacted to about 2×10 μg of ACh, *i.e.*, a concentration of about 2×10^{-7} g per ml. Similar results were reported by Hess *et al.* (1950). Quilliam's (1949) isolated iris preparation reacted to ACh concentrations of as small as 3×10^{-8} g per ml.

The observation that epinephrine and norepinephrine contract and serotonin relaxes the dilator muscle is in contrast with Apter's recent report (1960), which claims that in the cat "the so-called dilator pupillae is totally devoid of contractile power in any direction" and that contractile properties found in this area of the iris are due to ciliary body elements extending into the dilator area. We do not believe that our preparations contained any muscle fibers from the ciliary body as we took care to cut the dilator sectors within the iris and not in the area where ciliary body would be expected. Yet, the presence of ciliary elements cannot be excluded with certainty. It would be difficult, however, to conceive of adrenergic substances inducing contraction in elements which are mainly cholinergic, such as the muscles of the ciliary body.

Serotonin then induces a powerful contraction of the parasympathetically innervated iris sphincter and a weak, yet distinct, relaxation of the sympathetically innervated dilator muscle. On the sphincter of the iris, serotonin mimics the effect of the physiological transmitter of the cholinergic system. As to the intimate mechanisms involved in the serotonin action, the following considerations are in order: Principally, a drug can produce its effect upon an isolated parasympathetic organ *via* action at three different sites. 1) It may stimulate peripherally located ganglia. As the iris does not contain ganglion cells, we can dispose of this type of mechanism in our preparation. 2) The drug may trigger the release of transmitter substances. Such properties are well known only for the sympathetic but not the parasympathetic terminals. 3) We are led to

the third mechanism, namely, a direct action of the drug on the smooth muscle as the basis for these constrictive effects of 5-HT.

The relaxing effect of serotonin upon the dilator muscle is reminiscent of the dilating effect of isoproterenol on other adrenergically innervated organs, but our experiments offer no basis to identify further the intimate mechanisms involved in this action of serotonin. Neither can we suggest any explanation for the secondary contraction phase appearing with larger doses of serotonin.

SUMMARY

The effects of serotonin (5-HT) and a number of other substances were tested on the isolated intact cat iris and on the isolated iris sphincter and dilator muscle. The reaction of the pupillary opening on the intact iris was recorded by means of a photoelectric technique. The reaction of the isolated muscles was measured by means of ("isometric") mechanoelectric transducers.

The isolated iris reacted to serotonin with miosis. Concentrations of 0.3 μg per ml of Ringer solution were effective. The pupillary constriction and ensuing dilation lasted up to 15 and more minutes. Two-peaked patterns occurred often with larger doses. Serotonin treatment desensitized the preparation to later injections of 5-HT. The isolated constrictor muscle reacted to 5-HT with contraction; the dilator strip, with relaxation often followed by contraction when larger doses were used.

Epinephrine and norepinephrine induced dilation of the pupil. Norepinephrine led to contraction of the dilator muscle. Atropine was of small influence upon the serotonin reactions but in moderate doses eliminated the acetylcholine effect upon the intact iris and the constrictor muscle completely.

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