It can be seen by comparing the first columns in Tables 1 and 2 that both groups were comparable during the first clearance. The Saralasin infusion significantly decreased urine flow without affecting plasma osmolality and Na and K concentrations. Na excretion tended to decrease, but not significantly; the decrease in urine flow was counteracted by an increase in urinary Na concentration (the ratio urine/plasma Na increased but plasma Na was unaffected). In contrast, the ratio urine/plasma K concentration was not significantly increased, and this coupled with no significant change in plasma K, but a significant decrease in urine flow, resulted in a significant decrease in K excretion. Arterial blood pressure was unaffected.

DISCUSSION. Taken together with the observations made previously, our present results argue in favor of a direct renal effect of angiotensin II in goosefish. That is, angiotensin II administration resulted in a diuresis and administration of a competitive inhibitor of angiotensin II resulted in antidiuresis. In both cases changes in Na and K excretion rates tended to parallel the changes in urine flow, but the changes were not always significant. Changes in excretion rates cannot be ascribed to changes in blood pressure because although angiotensin II increased blood pressure (and urine flow), Saralasin decreased urine flow without affecting blood pressure.

Although there are many reports that exogenous angiotensin II produces diuresis in mammals (Ann. Rev. Pharmacol. 13:57-90, 1973 for several references) our results are the only indication that endogenous angiotensin, at normal levels, may have direct renal effects.

Our results are consistent with published reports that angiotensin II exerts a direct action on Na transport in the kidney (Am. J. Physiol. 212:1153-1157 [1967], Am. J. Physiol. 232:F298-F306 [1977]) and intestine (J. Physiol. 206:323-333 [1970], Am. J. Physiol. 224:1223-1228 [1973]). Because of the poor understanding of the mechanism by which urine is formed by the aglomerular fish, it is difficult to decide the precise mechanism of action of angiotensin II in this animal. It could stimulate entry of water and solute into, or inhibit reabsorption of water and solute from, the aglomerular tubules.

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## THE EFFECTS OF SODIUM PENTOBARBITAL ON HEMODYNAMICS AND RESPIRATION IN *S. acanthias*

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The effect of anesthetic agents during long term experimental procedures in poikilotherms is not well documented. In studies involving extensive surgery, the anesthetic most often used for fish has been MS-222 (tricaine methanesulfonate). The data of Stenger and Maren (Bull. MDIBL 7:51-55, 1967) and Peirce et al. (Bull. MDIBL 7:45-47, 1967) show this drug to be ineffective when given intravascularly and deleterious to the fish because of progressive depressant effects on cardiac output when added to the sea water. It is unsuitable for use in cardiovascular research. Sodium pentobarbital has also been used in poikilotherms. In the present study the effects of varying intravascular doses of sodium pentobarbital on the cardiovascular and respiratory systems of the dogfish are characterized.

Twenty male dogfish (1-2 Kg) were allowed to free-swim in a 0.3 $M^3$ tank supplied with running sea water. After a control period of at least one hour either saline (5 fish) or sodium pentobarbital (15 fish) were administered in the caudal artery. Doses of pentobarbital ranged from 2 to 164 mg/Kg. Opercular rate was counted visually at 10 minute intervals during the control period and for 4 hours following injection. From these data a dose-response curve was constructed.

A second group of 7 dogfish were prepared for recording hemodynamic parameters as described earlier (Bull. MDIBL 16:66-69, 1976). Arterial and venous blood samples were taken before and after sequential doses of sodium pentobarbital (3 mg/Kg, 9 mg/Kg, 30 mg/Kg) and were analyzed for hematocrit, $PO_2$ and pH (see

Bull. MDIBL 14:51-55, 1974). The gills were irrigated with 15°C sea water at a rate of 2l/min. Opercular rate was determined with the sea water flow turned off. In one fish given pentobarbital, a test of responsiveness of the gill vasculature was made by equilibrating the sea water to the gills with 5% $CO_2$ and eliciting a gill response to hypercapnia (Bull. MDIBL 8:35-38, 1968).

In three fish, blood gases were followed at hourly intervals for 4 hours, and the fish were exposed on sequential days to the following protocol: 1) free-swimming; 2) partially restrained in dorsal position with gills mechanically irrigated; 3) same as 2) but with 15 mg/Kg pentobarbital i.a. after first hour.

The effect of sodium pentobarbital on opercular rate is shown in Figure 1. There was no significant change in either the control fish or the fish given 4 mg/Kg. There was a marked drop in opercular rate after 28.0 mg/Kg. The composite of responses from 15 fish is shown in Figure 2. The lowest observed

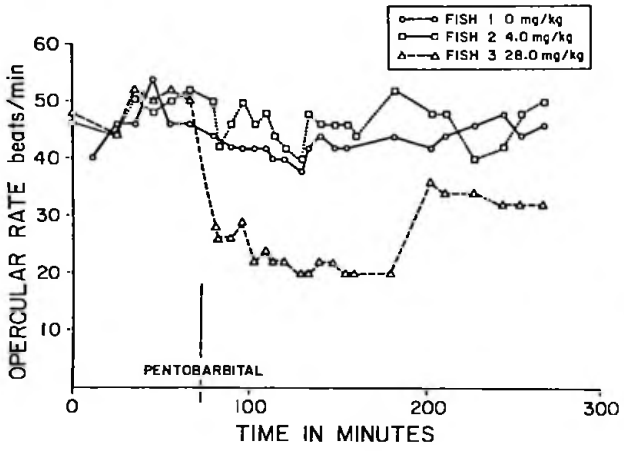


Figure 1. Opercular rate of three free-swimming fish vs time.

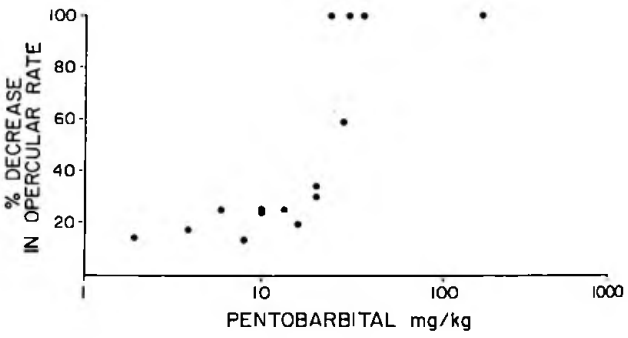


Figure 2. Dose-response curve for sodium pentobarbital in 15 free-swimming fish.

opercular rate after the time of injection was used to determine maximum change. The percent change in opercular rate of the fish receiving up to 8 mg/Kg pentobarbital did not differ from the control fish receiving saline. The lowest lethal dose was 24 mg/Kg.

The response of several hemodynamic variables to 3 and 30 mg/Kg pentobarbital are shown in Figure 3. These results are typical of the seven instrumented fish studied. There was very little effect of the lower dose on any of the variables. A paired t-test comparing values before and after 3 mg/Kg pentobarbital on seven fish revealed no significant difference in arterial $PO_2$, pH, opercular rate, heart rate, gill resistance or oxygen consumption. In fish given 9 mg/Kg pentobarbital heart rate increased and opercular rate dropped slightly. With higher doses, 30-40 mg/Kg, heart rate increased significanlty 19.7 ± 1.2 to 26.3 ± 1.2) and opercular rate dropped (35.5 ± 5.4 to 23.0 ± 8.5). There were no significant changes in $PO_2$ or pH after pentobarbital but there was a 25% fall off in oxygen consumption attributable to the decrease in cardiac output with a 34% decrease in stroke volume. The effect of hypercapnia can also be seen in Figure 3. Driving pressure across the gills increased with bradycardia and lowering of cardiac output. The net effect was an increase in gill resistance. In three fish, measured arterial $PO_2$ and pH did not change over a four hour time period. Blood gases were the same whether the fish were free-swimming, partially restrained, in a dorsal position, or dosed with 15 mg/Kg pentobarbital and in a dorsal position.

Sodium pentobarbital in doses between 10 and 20 mg/Kg effectively reduces opercular rate in dogfish without having much influence on $PO_2$ or pH. Presumably these doses, in the intact fish, have little effect

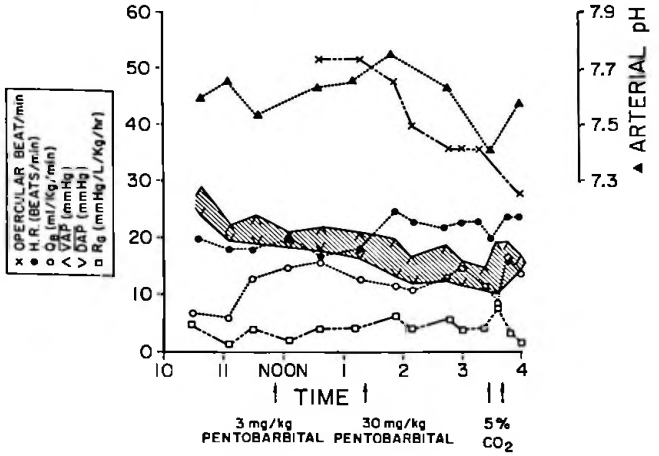


Figure 3. Effects on hemodynamic parameters of 3 mg and 30 mg/Kg pentobarbital followed by hypercapnia. Fish partially restrained and gills artificially irrigated.

on oxygen consumption or cardiac output. Higher doses are lethal unless the gills of the fish are artificially irrigated. Doses lower than 10 mg/Kg have no effect on any of th e parameters measured in this study. It is questionable that doses below 10 mg/Kg are effective at all.

In artificially irrigated fish, large doses of sodium pentobarbital (30 mg/Kg) have a slightly depressant effect on cardiac output and oxygen consumption, but the cardiovascular responsiveness of the animal is not interrupted as demonstrated by the reflex response to hypercapnia. There is, however, a demonstrable uncoupling of the usual cardio-ventilatory interaction. High doses of pentobarbital lead to tachycardia which is probably due to a decrease in vagal inhibition secondary to CNS depression. The concomitant decrease in opercular rate could be explained either as a central, medullary depressant effect, or again as a decrease in vagal inhibition. It has been postulated that vagal afferent, similar to Hering-Breuer stretch receptors in mammalian systems, operate in the fish gill to control opercular rate (Nature 211: 1187-1188, 1966). A direct effect on the vagus could then increase heart rate and inhibit the opercular rate.

The duration of action of the sodium pentobarbital is related to the size of the dose. The range between the dose which effectively changes opercular rate and the lethal dose is narrow. Pentobarbital in doses between 10 and 20 mg/Kg does not show the progressive deleterious effects found with MS-222. That sodium pentobarbital has any anesthetic effect in this range is doubtful, and it has a minimal effect on cardiovascular and metabolic systems. This project supported by Veterans Administration Hospital, Bronx, New York, Project numbers 4901-01 and 4901-02 and NIH General Research Support Grant for Mount Desert Island Biological Lab (#5 507RR05764) and the Atwater Kent Foundation.

## A METHOD FOR DETERMINING RELATIVE FUNCTIONAL SURFACE AREA OF THE DOGFISH GILL

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Although neural and hormonal stimuli cause vasomotion of the gill vasculature the effect on the respiratory exchange area is not clear. A change in resistance in gill vasculature can be estimated from the ratio of pre- and post gill pressure and cardiac output. When cardiac output is maintained constant, a change in driving pressure across the gills reflects a change in resistance. Because of the parallel