Potentiation of Pentobarbital Anesthesia by Competitive Protein Binding

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THE POTENTIATION of barbiturate anesthesia should be of interest to the experimentalist working with drugs capable of inducing convulsive activity. This is particularly true when the excitatory drug itself shows the peculiar paradoxic property of potentiating barbiturate anesthesia. In this circumstance an increase in the circulating quantity of a convulsant may be tolerated beyond ordinary threshold levels by virtue of a concomitant sustained increase in the effective anticonvulsant barbiturate levels.

In this report we show that Urokon (sodium acetrizoate–Mallinckrodt), a contrast material utilized in roentgen angiographic studies and known to produce convulsive phenomena when injected in large quantities in experimental animals, may potentiate sodium pentobarbital (Nembutal) anesthesia and thereby induce an additional measure of protection against its own central nervous system effects.

Recognition of barbiturate potentiation by contrast media injections has appeared previously in the literature. In 1956, McAfee and Willson [1] stated: ". . . the duration and depth of depression after aortography under Pentothal are greater than is usually seen in surgical patients receiving a similar amount of Pentothal." In 1959, Killen, Lance, and Owens [2] noted incidentally that the animals under pentobarbital anesthesia and receiving Urokon showed potentiation of anesthesia.

Methods

Stock white female Wistar rats of average adult weight (approximately 250 grams), maintained on standard Wayne Lab Blocks, were used throughout the experiments.

(1) Twenty rats were anesthetized by intraperitoneal injection of 6 per cent pentobarbital at a dosage of 36 mg./kg. Here, as in the remainder of the experiments, the rats were observed for a period of time elapsing from the onset of anesthesia to the first indication of wakefulness in response to either finger snapping against the side of the cage or to ear pinching. Eye opening, whisker twitching, and spontaneous movements were generally the first signs of wakefulness.

(2) Twenty rats were injected with the same dosage of pentobarbital but received in addition intramuscular 70 per cent Urokon at a dosage of 4,900 mg./kg. (7.0 ml./kg.)

(3) Twenty-two rats were injected with both pentobarbital and Urokon as outlined, but received in addition 15 per cent Rheomacrodax, a low molecular weight dextran preparation, intraperitoneally, at a dosage of 10.0 ml./kg.

(4) Control groups for duration of anesthesia were injected in the dosages already noted with (a) pentobarbital and normal saline (7.0 ml./kg.), (b) pentobarbital and hypertonic (7.0 per cent) saline (7.0 ml./kg.), and (c) pentobarbital and Rheomacrodax (10.0 ml./kg.). As a further control series for the pentobarbital-Urokon-Rheomacrodax experiment, a group of rats were observed for duration of narcosis after substitution of 5 per cent dextrose in water for the Rheomacrodax (10.0 ml./kg.).

(5) The effect of varying the dosage of Urokon in the pentobarbital-Urokon-Rheomacrodax narcosis potentiation experiment was determined. Dosages for pentobarbital and Rheomacrodax were maintained at the rates indicated above, but in separate groups of rats the Urokon dosage was diminished to ½, $\frac{1}{10}$, and $\frac{1}{100}$ of the original dosage level.

(6) The duration of narcosis was determined in 19 rats anesthetized with pentobarbital as above but with 50 per cent sodium

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TABLE 1. Urokon Potentiation of Pentobarbital Narcosis

	No. of Rats	Duration of Narcosis (minutes)		*P*-Value Significance of Difference from Pentobarbital Mean
		Range	Mean ± S.E.	
6% Pentobarbital I.P. 36 mg./kg.	20	75–364	226 ± 15.6	—
6% Pentobarbital I.P. 36 mg./kg. and normal saline I.M. 7.0 ml./kg.	17	144–339	246 ± 16.0	>0.3
6% Pentobarbital I.P. 36 mg./kg. and 70% Urokon I.M. 4,900 mg./kg.	20	213–564	374 ± 14.9	<.001
6% Pentobarbital I.P. 36 mg./kg. and 70% Urokon I.M. 4,900 mg./kg. and 15% Rheomacrodax I.P. 10 ml./kg.	22	464–746	571 ± 14.6	<.001

diatrizoate (Hypaque) substituted for Urokon in equal molar quantities (5,500 mg./kg.). The same was done with substitution of 52 per cent sodium and methylglucamine iodipamate (Cholografin) (13,520 mg./kg.).

(7) A group of rats injected with Urokon at 4,900 mg./kg. dosage was placed in a common chamber with rats injected with normal saline, with controlled atmospheres of ether. Total sleep time was determined for each group.

(8) Urokon was injected in the lethal dose range (16,100 mg./kg. of 70 per cent Urokon, intraperitoneally) in (a) a group of 20 rats without other drugs, (b) 20 rats injected with 10.0 ml./kg. of 15 per cent Rheomacrodax, intraperitoneally, (c) 18 rats injected with 6 per cent pentobarbital at a dosage of 36 mg./kg., intraperitoneally, and (d) 19 rats injected with both pentobarbital (36 mg./kg.) and Rheomacrodax (10 ml./kg. of 15 per cent solution) as noted.

(9) The Urokon-pentobarbital narcosis potentiation studies were repeated with rabbits. The dosages used were 36 to 42 mg./kg. of pentobarbital and 3,500 to 4,900 mg./kg. of Urokon.

Results

It is apparent from table 1 that there was a statistically significant prolongation of anesthesia noted in rats injected with Urokon as well as pentobarbital in comparison to rats injected with pentobarbital alone or pentobarbital with saline. It is also apparent that rats injected with pentobarbital and Urokon, and

TABLE 2. Duration of Survival After Injection of Lethal Dose Urokon

	No. of Rats	Duration of Survival (minutes)		*P*-Value Significance of Difference from Urokon Group
		Range	Mean ± S.E.	
70% Urokon I.P. 16,100 mg./kg.	20	40–173	74 ± 9.4	—
70% Urokon I.P. 16,100 mg./kg. and 15% Rheomacrodax I.P. 10 ml./kg.	20	60–113	79 ± 9.4	>0.6
70% Urokon I.P. 16,100 mg./kg. and 6% Pentobarbital I.P. 36 mg./kg.	18	52–210	156 ± 9.3	<.001
70% Urokon I.P. 16,100 mg./kg. and 6% Pentobarbital I.P. 36 mg./kg. and 15% Rheomacrodax 10 ml./kg.	19	20–240	205 ± 9.3	<.001

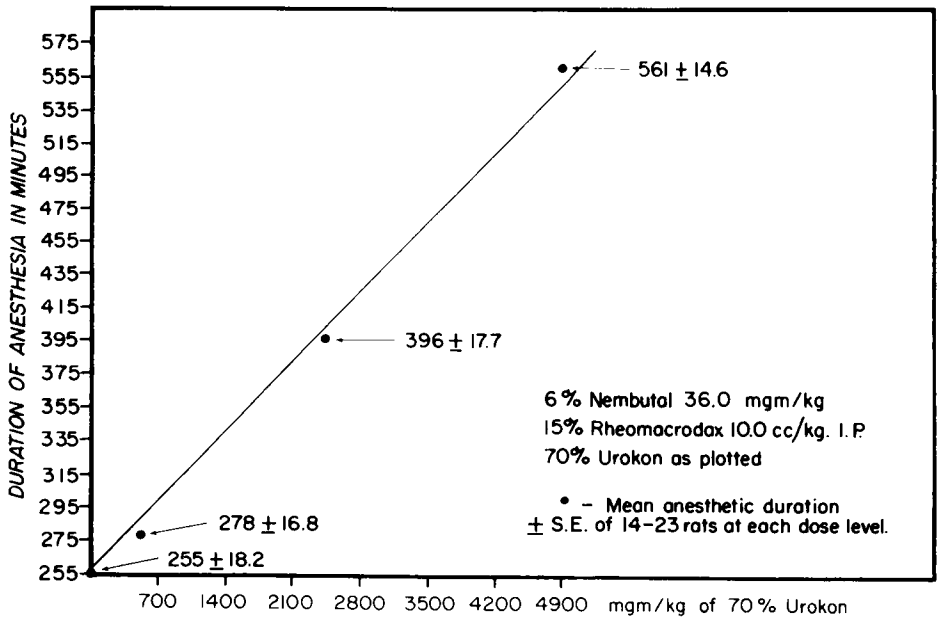

FIG. 1. Effect on potentiation of pentobarbital (Nembutal) narcosis by increasing dosages of Urokon in Urokon-Rheomacrodax combinations.

further injected with Rheomacrodax showed an even more marked prolongation of pentobarbital narcosis. It should be pointed out at this time that rats under pentobarbital anesthesia (without Urokon) and injected with either Rheomacrodax or 7 per cent saline, a solution isosmotic with 70 per cent Urokon, failed to show any prolongation of total sleep time. The mean duration of narcosis for the pentobarbital-Rheomacrodax group was 210 ± 16 minutes, and for the pentobarbital-7 per cent saline group, 151 ± 9.1 minutes. Furthermore, the substitution of 5 per cent dextrose in water for Rheomacrodax was ineffectual in prolonging the pentobarbital-Urokon narcosis (Mean 384 ± 18.7 minutes in 13 rats.)

Figure 1 indicates the almost linear increase in mean anesthesia duration in rats injected with constant dosages of pentobarbital and Rheomacrodax but with varying amounts of Urokon.

When the dosage of Urokon was increased to the lethal range (16,100 mg./kg.), all rats expired within a four-hour period (table 2). The addition of Rheomacrodax alone did not increase immediate survival time; but the group of rats receiving pentobarbital in addition to the Urokon, and the group receiving both pentobarbital and Rheomacrodax as well as the Urokon, showed a distinct prolongation of survival times. The protection apparently conferred on these latter two groups of rats in the post-injection period appears to parallel the relative potentiation of narcosis previously noted.

Hypaque injected in molar dosages equivalent to Urokon failed to potentiate pentobarbital narcosis. (Mean anesthesia duration 220 ± 22.0 minutes.) Cholografin, on the other hand, when injected in dosages equivalent to Urokon in rats under pentobarbital anesthesia, proved quite toxic. Twenty rats tested in this fashion died of the injection within 48 hours, but it is significant that no rat awakened from anesthesia during this period and approximately half of the rats continued under narcosis for 24 hours or longer. Cholografin dosage was then cut in half and the experiment repeated. Under these circumstances, the average sleep time in 20 rats was 340 ± 65.5 minutes, which is almost equivalent to that noted with injections of twice the equivalent molar quantity of Urokon. When rabbits were

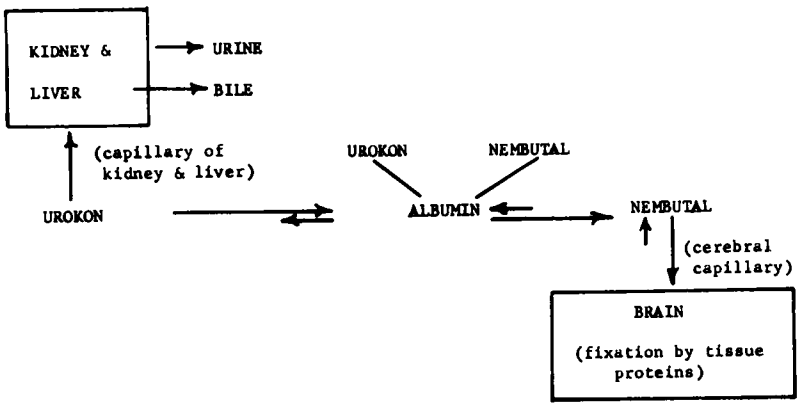

FIG. 2. Hypothesis of potentiation.

substituted for rats, no potentiation of pentobarbital narcosis could be achieved by injections of Urokon. Rabbits anesthetized under these circumstances awakened at the same average time period as did the controls injected with normal saline.

No potentiation of ether anesthesia was produced by injection of Urokon. Ten rats injected with Urokon in a common ether chamber with control saline-injected rats awakened from ether anesthesia at approximately the same average time as did the latter group.

Discussion

Urokon, a roentgen contrast material that, until recently, has been employed widely in clinical radiology, is one of a number of tri-iodobenzoic acid compounds that have served primarily for angiography and intravenous pyelography. In recent years, it has been supplanted by less toxic tri-iodobenzoic acid compounds. In all species tested, the major pathway of elimination of Urokon is via the kidneys (chiefly by glomerular filtration). A second, but considerably less important pathway of excretion, is found in the bile.[3, 4] Hypaque is another contrast material that has been utilized for angiographic and pyelographic studies. Like Urokon, it is rapidly excreted via the glomeruli, and to a slight extent via bile.[5] In recent years considerable evidence [6, 7, 8] suggests that the tri-iodobenzoic acid compounds (such as Hypaque) with completely substituted benzoic acid rings are less toxic than those with one or more unoccupied positions on the ring (such as Urokon).

Cholografin, the third contrast material utilized in this study, consists essentially of two Urokon molecules joined back-to-back by acetamido chains, with an ethyl group interposed. It has a greater protein binding capacity than Urokon and is correspondingly more toxic.[8] The major pathway of excretion is via the liver, with variable small amounts appearing in the urine.

A host of substances have been reported to potentiate barbiturate anesthesia in the experimental animal. Among these are a number of sedatives, hypnotics, and tranquilizers, but also many other compounds that do not lend themselves readily to additive depression.[9, 10, 12-15] The mechanism of barbiturate potentiation with drugs that do not produce central nervous system depression has been obscure. There has been no substantial evidence to support such explanations as altered osmotic activity, altered permeability, or additive uncoupling of oxidative-phosphorylation.

There is, however, general agreement that only freely diffusable, unbound pentobarbital can pass the "blood-brain barrier." [13, 16, 17] Once beyond the "blood-brain barrier," narcosis may depend on the percentage of the unbound drug that passes into intracellular sites and undergoes binding to tissue protein.[17, 18] Cognizance must be taken of the dependence of binding on *p*H,[8, 16, 17] and of diffusability upon lipoid solubility.[19]

With these considerations in mind, an hypothesis can be formulated for potentiation of pentobarbital anesthesia that seems compatible with the data we have compiled. Under this

hypothesis (fig. 2), potentiation is achieved by the unfavorable competitive position of pentobarbital with Urokon for protein binding sites in hepatic and renal excretory cells and in circulating plasma. This eventuates in both sustained elevations of total plasma pentobarbital levels secondary to diminished conjugative and excretory mechanisms, and in increased amounts of *unbound* pentobarbital available to the tissues including the central nervous system.

To support this hypothesis, it is necessary first to discredit the alternative possibilities alluded to in an earlier section; namely, that potentiation might be the result of (a) additive central nervous system depression, (b) altered osmotic factors, (c) altered permeability factors.

In regard to additive central nervous system depression, it should be noted that the control rats injected with Urokon alone consistently manifested an excitatory, hyper-irritable state rather than depression, and when the Urokon dosage was sufficiently increased, the hyper-irritable state proceeded to convulsive activity. Furthermore, potentiation of anesthesia was not noted when ether was used for narcosis rather than pentobarbital.

Changes in osmotic factors are likewise unlikely. While all of the contrast media utilized in the study were hypertonic, they were injected in equal molar quantities intramuscularly, and the average injection was not greater than 2 to 3 ml. Furthermore, control studies with saline solutions isotonic with 70 per cent Urokon (7.0 per cent saline) failed to prolong pentobarbital narcosis.

Insofar as increased permeability is concerned, no evidence for this could be found in rats studied with a trypan-blue indicator or by utilization of I^{131}-labeled Urokon. No staining of the brain itself could be noted in 5 rats given 5 ml. of an 0.1 per cent trypan-blue solution intraperitoneally several hours prior to potentiation of Urokon-pentobarbital. When brain counts were made in groups of rats sacrificed at varying intervals after injection of pentobarbital and I^{131} Urokon, no more than an average of 0.5 per cent of injected activity could be detected in the brains.

The evidence in support of pentobarbital potentiation by the postulated system of competitive protein dynamics is as follows:

(1) Both Urokon and pentobarbital to a considerable extent are bound to albumin at physiological *p*H values.

(2) Competitive equilibrium dialysis has indicated that, at the calculated *in vivo* concentrations of pentobarbital and Urokon, pentobarbital does not effectively displace I^{131} Urokon from its albumin-binding sites. With considerable increase in pentobarbital-Urokon ratios (100:1), however, there is significant displacement, suggesting that these substances do compete for similar binding sites.[20]

(3) Cholografin, shown previously to be a better binder than Urokon, increased the potentiation of pentobarbital narcosis to a greater extent than did Urokon.

(4) Hypaque, a poorer binder than Urokon, did not appreciably alter pentobarbital narcosis over control values.

(5) When rabbits were tested rather than rats, no potentiation of pentobarbital narcosis developed on injection of Urokon. The advantage of the rat over the rabbit, in studies on Urokon binding potential, has been noted earlier.[6]

Before accepting these arguments, however, one must seek an explanation for the enhanced potentiation noted with low molecular weight dextran. The decision to study the effects of the low molecular weight dextran preparation originated in a review of the work of Bernstein and Evans,[21] who utilized this preparation favorably to modify the acute toxicity syndrome following the intravenous injection of high dosages of 90 per cent Hypaque in dogs.

Previous work in our laboratories had failed to demonstrate any alteration in protein binding dynamics of the contrast media in the presence of dextran. Furthermore, the almost linear relation between increased sleep time and increasing dosage of Urokon in the rats pre-treated with pentobarbital and Rheomacrodax suggested that the Urokon levels alone constituted the controlling factor in pentobarbital potentiation. The logical expectation, then, was that pre-treatment with Rheomacrodax altered excretion of Urokon in a direction that led to sustained maintenance of higher

Urokon plasma levels in comparison to control animals.

This was confirmed by determining the two-hour post-injection urinary excretion of I^{131}-labeled Urokon in 10 rats pre-injected with both pentobarbital and Rheomacrodax, in comparison to 10 rats pre-injected with pentobarbital alone. The group pretreated with both pentobarbital and Rheomacrodax showed an average of 7.8 per cent of the injected Urokon in the urine and 2 per cent in intraperitoneal fluid aspirates at this period, in comparison to an average of 15 per cent in the urine (and no gross peritoneal fluid) in the Urokon controls pre-injected only with pentobarbital.

The mechanism of the diminished excretion of Urokon in the urine of the Nembutal-Rheomacrodax animals remains obscure. It seems unlikely that osmotic factors played a predominant role since only 2 per cent of the injected I^{131} Urokon was recovered in peritoneal aspirates at the time of sacrifice, and the total urine volumes in the two groups of animals did not differ significantly. Perhaps the observations made by Theile and Theile [22] have some bearing on the situation. Colloids such as dextran or PVP, when given as infusions to premature infants to counteract hyperbilirubinemia, appeared to drive albumin out of the blood stream and into the interstitial spaces.

The question of barbiturate protection against injected contrast media has been touched upon in two previous reports. In 1950, Ziperman, Hughes, and Shumacker [23] pointed out that pentobarbital and other barbiturates in proper dosages conferred a considerable degree of protection against the lethal effects of Diodrast in the mouse. In 1960, Hoppe and Archer [24] reported that rabbits, cats, and dogs under pentobarbital anesthesia were able to tolerate from 20 to 800 per cent more contrast media (Hypaque, Urokon, Neo-Iopax, Diodrast) on intracisternal injection than unanesthetized controls.

The present study points up the parallel relationship between potentiation of pentobarbital anesthesia and the duration of conferred protection against large doses of Urokon. Work currently under way in our laboratories suggests that the potentiation of anesthesia is manifested by sustained pentobarbital brain levels, and under these circumstances, it is not surprising to find a parallel continued protection against the excitatory effects of Urokon.

Summary

Several opaque media used in clinical radiology extend the duration of pentobarbital anesthesia in rats. A number of experimental findings appear to link pentobarbital potentiation to the protein binding propensity of the potentiator. Thus, the ability of a contrast material to usurp albumin binding sites of pentobarbital on *in vitro* competitive equilibrium dialysis is a reflection of *in vivo* competition for similar sites on plasma proteins. This in turn reflects the amount of unbound, freely diffusable pentobarbital available to the CNS at any given moment.

Since toxic dosages of the contrast media produce convulsive activity and pentobarbital affords a degree of protection against such phenomena, the potentiation of pentobarbital might be expected to modify the toxicity of contrast material injections but in a misleading fashion. The significance of these findings are discussed in the text.

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INERT-GAS ANESTHESIA Work has been done in establishing the biological role of xenon and other rare gases. Xenon may be concerned with the postulated mechanisms of anesthesia. Correlation between the abilities of many substances to produce anesthesia and their solubilities in lipoid materials has diverted the attention of many investigators in the field of the mechanisms of anesthesia for over 50 years. Recent re-emphasis that water, or proteins, or both may have a significant role in the mechanism of anesthesia suggests that no one property determines the anesthetic potency of a given substance. With the introduction of an anesthetic molecule into the lung alveoli, many processes must occur between this site and the final site of action. A different set of chemical and physical properties would be required of the anesthetic for each of these processes to operate under optimal conditions. Possession of any one property by a molecule may be a deterrent, an advantage, or immaterial to its ability to produce anesthesia in the intact organism. (*Featherstone, R. M., and Muehlbaecher, C. A.: Current Role of Inert Gases in the Search for Anesthesia Mechanisms, Phar. Rev. 15: 97 (Mar.) 1963.*)