THE EFFECT OF VARIOUS TISSUES ON THE DETOXIFICATION OF EVIPAL IN THE DOG

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The ultra-short action of evipal-sodium (salt of cyclohexenylmethyl-N-methyl barbituric acid) [1] has interested many investigators in regard to the tissues concerned in its inactivation. The original contention of Weese (1) that evipal is destroyed by the liver has also been suggested by Cameron and deSaram (2). The conclusion was made essentially upon the prolongation of evipal action following preliminary injury to the liver by various agents. Since the decrease in function of the liver was not determined, its full significance was not evaluated in their studies or subsequent ones. Furthermore, except for the kidney (3), the role played by other tissues has not been investigated. An attempt was made in this study to establish more clearly the significance of the liver and other tissues in the destruction of evipal.

Procedure and Results

Adult dogs were used, three (3) having been prepared for blood pressure recording with a Van Leersum carotid loop. Evipal was administered intravenously in all cases at a rate of 4 cc. per minute, the narcotic dose being accepted as 30 mg. per kilogram (1). All solutions

TABLE 1

Effect of Optimum Concentration of Evipal (5%) Given Intravenously in Dogs

No. of Dogs	Deep Sigh	Maximum Variations During Anesthesia			During Depth of Anesthesia					Recovery
		Blood Pressure	Resp. Rate	Heart Rate	Lid Reflex	Wink Reflex	Response to Pain	Muscle Tone		
								Jaw	Skeletal	
	sec.	mm. Hg	min.	min.						min.
19	35 (20–50)	132/86 to 70/40 to 120/90	32 to 8 to 38	102 to 166 to 90	Markedly depressed or absent	Moderately depressed	Absent	Moderately decreased	Markedly decreased or absent	98 (53–170)

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were freshly prepared. At approximately 5 minute intervals, records were made of various physiologic phenomena noted in Table 1. Pain was initiated by clamping the muco-cutaneous region of the anus. Recovery or end of anesthesia was regarded as the moment when the dogs showed normal reflexes and were able to support their body weight.

Control studies on 19 dogs were carried out to determine the optimal concentration of evipal following intravenous administration. The concentrations of evipal used were 2.5, 5 and 10 per cent. It was established from 43 observations that a 5 per cent. concentration of evipal gave consistent results with respect to the depth, duration and recovery of anesthesia and was therefore considered an optimal concentration. In Table 1, the results showing average responses with this concentration are briefly summarized. While the respirations decreased progressively in depth, there was an initial but temporary increase in minute volume followed by a fall. The blood pressure generally showed an acute and transient fall which was prevented (5 experiments) when a preliminary intramuscular injection of ephedrine sulphate (1.5 mg./kgm.) was given. While ephedrine did not delay the onset of anesthesia, it did shorten the duration. With the dosage used, the wink reflex was only moderately depressed but was exceptionally absent. Anesthesia appeared in about one minute after the injection and remained moderately uniform in depth for about 20–25 minutes. Recovery was noted at an average of 98 minutes.

Hepatic damage was induced by chloroform anesthesia of 1½–2 hours duration in 5 control dogs previously fasted for 16–18 hours. The extent of injury was determined 48 hours later by the bromsulphalein method (4) and compared to control values. The plasma dye retention after 30 minutes in dogs with liver damage ranged between 45–80 per cent.; the control values averaged 4.1 per cent. A full narcotic dose of evipal was given to 3 dogs and ½ of the dose to two others. Table 2 summarizes the results. The death of one dog and the mark-

TABLE 2

EFFECT OF EVIPAL IN DOGS WITH LIVER INJURY AND FOLLOWING EVISCERECTOMY

Condition of Dogs	No. of Dogs	Narcotic Doses of Evipal 30 mg./kgm.	Recovery	Remarks
			min.	
Liver Injury by Chloroform	3	Full dose	(290),* 240, 290	*Died
	2	½ dose	172, 110	
Eviscerectomy	3	Full dose	(30),* (67),* (240)*	*All died
	2	½ dose	150, 203	Slight muscle tone
	1	⅓ dose	120 (177)*	*Died after 2nd injection

edly prolonged anesthesia in three others suggests the importance of the liver in the detoxification of evipal and confirms previous findings (1, 2).

Hepatic function, however, was not completely eliminated by the chloroform anesthesia and its significance therefore not fully revealed. Accordingly, in six other control dogs, the liver and most of the gastro-intestinal tract were removed under light ether anesthesia (40–60 min.) (5). The animals were maintained for 6–17 hours with periodic intravenous injections of 10 per cent. glucose solution. Blood sugar determinations (6) were frequently made and ranged in value from 122–510 mg. per cent. Three to five hours after operation and apparent recovery noted by the dog's actions and ability to walk, evipal was administered in varying doses depending on the post-operative weight. The depth and duration of anesthesia were recorded.

TABLE 3

EFFECT ON DOGS OF EVIPAL SOLUTION AFTER INCUBATION WITH VARIOUS RAT TISSUES

No. of Observations	Tissue Used	Amt.	5% Evipal in Sal.	Time of Incub. 38° C.	During Depth of Anesthesia			Recovery	Approximate Destruction Evipal
					Lid Reflex	Wink Reflex	Skeletal Muscle Tone		
		gms. or cc.	cc.	min.				min.	%
9	Control	—	15	60	Markedly depressed or absent	Moderately depressed	Markedly depressed	109 (90–184)	—
16	Liver	9 (5–14)	21 (10–40)	30 (10–60)	Slightly depressed	Normal	Very slight decrease	36 (1–57)	66
12	Skeletal Muscle	11 (9–14)	25 (20–30)	30	Slightly depressed	Normal	Moderately decreased	57 (38–65)	48
8	Spleen	10 (7–14)	22 (15–30)	30	Moderately depressed	Normal	Moderately depressed	68 (46–80)	38
10	Kidney	8 (5–14)	22 (14–32)	45 (30–60)	Absent	Moderately depressed	Absent	96 (80–119)	0
6	Brain	2	10	30	Absent	Moderately depressed	Markedly depressed	116 (92–126)	0
6	Oxalated Dog Blood	20	1*	30	Absent	Markedly depressed	Absent	134 (79–192)	0

* 1 gram of crystals of Evipal Sodium.

It will be seen from Table 2 that the full narcotic dose of evipal is fatal in eviserectomized dogs and that death occurs much sooner than in those dogs with liver injury due to chloroform anesthesia. Dogs receiving one half the dose recovered with normal lid and wink reflexes and pain response, but with only a slight return of muscle tone. Two

injections of ⅓ of the dose were given to one dog. Full recovery was noted in 120 minutes after the first administration. Death followed in 77 minutes after the second. Control dogs receiving ½ or ⅓ of the narcotic dose recovered in about 51 and 28 minutes respectively.

The fact that eviscerectomized dogs, receiving subnarcotic doses of evipal, recovered would seem to indicate that other tissues than the liver played a part in the detoxification of evipal. To determine this, a series of in vitro experiments were performed. Freshly macerated tissues of the rat were put in evipal solution made up to 5 per cent with physiological saline and incubated at 38° C. for 10 to 60 minutes (average 30 minutes). Some difficulty was experienced in getting clear solutions of macerated liver and spleen. After centrifuging, the fluid was drawn off and administered intravenously to dogs at 4 cc./min. with a dose of evipal of 30 mg./kgm. Control injections were made with incubated 5 per cent. evipal solution in saline. The reactions and duration of anesthesia of each dog were noted.

Table 3 shows the average responses of dogs after injections of evipal previously incubated with various macerated rat tissues and oxalated dog blood. It would appear that liver, skeletal muscle and spleen exert a significant action in nullifying the depressant action of evipal. The magnitude of such effect is in the order of the tissues named. In the case of liver, the duration of narcosis was decreased 15 per cent. after 10 minutes' incubation, 66 per cent. after 30 minutes and 90–100 per cent. after 60 minutes. Dogs in the last group showed nausea, emesis, mild excitement but no narcosis. After incubation with macerated muscle or spleen for 30 minutes, evipal anesthesia was shortened about 48 and 38 per cent. respectively. No decrease of the time of anesthesia was noted with evipal solution incubated alone or with rat kidney, brain or whole dog's blood. The negative results obtained with macerated kidney and brain tissues appear to minimize the factor of dilution in these incubation experiments.

DISCUSSION

In the preliminary phase of this study, attention is called to the fact that a 5 per cent. evipal solution is the optimal concentration for narcosis in dogs when given intravenously at the rate and dose used. The signs noted during the depth of anesthesia as well as the duration of anesthesia were significantly constant.

According to Weese (1), the minimal narcotic dose of evipal sodium (30 mgm./kgm.) given intravenously in dogs is approximately ⅓ of the minimal lethal dose (100 mg./kgm.). The data in this study indicates that the same narcotic dose produces an abnormally prolonged anesthesia in dogs with hepatic injury and death in maintained eviscerectomized animals. The liver thus plays a major role in the destruction of evipal. This contention is further brought out by the incubation studies.

However, the fact that eviscerectomized animals receiving 33 or 50 per cent. of the narcotic dose lived at all and showed normal or prolonged anesthesia followed by recovery suggests that other tissues may detoxify evipal or that it may be excreted rapidly. Such detoxification of evipal is also suggested by the incubation studies in which muscle and spleen shortened the narcotic action significantly. Hence, while the liver is the major site of detoxification, it is aided greatly by skeletal muscle and the spleen. The brain, kidney and blood apparently do not decrease evipal action.

Although the incubation experiments have shown that various tissues can destroy evipal sodium, no quantitative measure of such activity has been made in the living animal. The findings are therefore only qualitatively important. Furthermore, the actual mechanism involved in decreasing the action of evipal has not been revealed in these studies. The evipal may be adsorbed as such, conjugated in the various tissues, or excreted in small amounts in the urine unchanged (1).

CONCLUSIONS

Studies on eviscerectomized dogs and with incubated macerated tissues revealed more clearly the important role played by the liver in the inactivation of evipal. Skeletal muscle and spleen also shared in this action. Brain, kidney and blood had no effect on the destruction of evipal soluble.

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