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Feeding Behavior in Sheep as Related to the Hypnotic Activities of Barbiturates Injected Into the Third Ventricle

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SEOANE, J. R. AND C. A. BAILE. *Feeding behavior in sheep as related to the hypnotic activities of barbiturates injected into the third ventricle.* PHARMAC. BIOCHEM. BEHAV. 1(1) 47–53, 1973.–The central effect of barbiturates on feeding behavior was studied in sheep. Control or test solutions (0.5 ml) were injected at a rate of 0.19 ml/min into the third cerebral ventricle. Barbital, phenobarbital, amobarbital and pentobarbital elicited marked feeding ($p<0.01$) while secobarbital and thiamylal did not. The dose of barbital required to elicit maximum feeding was 240 μmoles/animal while for pentobarbital it was 30 μmoles. At these doses, barbital elicited a larger feeding response than pentobarbital (753±184 g vs. 394±178 g. 2 hr postinjection, $p<0.01$). No differences were observed between barbiturates that elicited feeding with respect to the latency of the response, within 2 min postinjection. Pentobarbital, but not barbital, increased intraperitoneal temperature ($p<0.01$), suggesting that pentobarbital had penetrated into brain structures involved in temperature regulation. The feeding responses observed may be related to the differences between barbiturates with respect to their lipid solubility and rate of penetration of biological membranes.. Since barbiturates are neurodepressants, we suggest that they act by partially removing the influence of inhibitory neurons impinging on feeding centers of the lateral hypothalamus.

Feeding behavior Temperature regulation Barbiturates Hypnotics

SEVERAL anesthetics have been injected centrally to test their effect on feeding behavior of mono and polygastric animals. Injections of pentobarbital into the lateral ventricles of rats increased their feed consumption [3,15]. Similar injections of pentobarbital caused feeding in goats, sheep and calves [1,19] and increased rumination in sheep [20]. Other anesthetics such as chloral, chloralose and magnesium chloride induced feeding in cats [8]. While the exact mode of action of these drugs remains unclear, the feeding response observed is probably related to their hypnotic properties. These drugs, when injected into the cerebrospinal fluid, presumably diffuse into adjacent structures, including the ventromedial hypothalamus, causing a decrease in neuronal activity. This could suppress medial inhibitors of the lateral hypothalamus, thus resulting in feeding.

The purpose of the present experiments was to study the effect of barbiturates on feeding behavior of sheep. The available barbiturates vary widely in their hypnotic properties, from the ultrashort acting thiamylal and thiopental to the long acting barbital [16]. Since barbiturates differ in their activities and degrees of solubility in the lipid fractions of the membranes [4,14], their effect on the feeding response may also differ. We injected these drugs into the third cerebral ventricle, to reduce their action in higher brain structures and produce larger concentrations of the drugs localized especially in the medial diencephalon, where their postulated action for feeding takes place.

METHOD

Fifteen yearling crossbred wethers averaging 40 kg of body weight were surgically implanted with stainless steel guides directed towards the third ventricle of the brain following the technique described by Seoane and Baile [21]. The final position of the tip of the guide was approximately 5 mm above the third ventricle. A 21 gauge needle was inserted through the guide into the third ventricle at the time of injections. The length of the inserts were determined during surgery. Post surgical treatment included antibiotic administration during five days. The animals were housed in behavioral rooms with controlled environmental conditions of temperature (21 ± 1°C) and relative humidity (45%). Water was available at all times. Sheep were fed ad lib. Fresh feed was offered one hour before the injections. The feed was a pelleted mixture of

60% concentrate and 40% hay. The gross composition of the feed has been described previously [22].

The injections of either control solutions (saline or synthetic CSF [18]) or the test drug were conducted at the same hour of the day for each experiment. The patency of the third ventricular insert was determined prior to the injections by the ease of injection and withdrawal of CSF using a column of saline in translucent tubing placed 20 cm above or 20 cm below the tip of the intraventricular insert. Half a ml of the test solutions was injected in 160 sec (approx. flow rate: 0.19 ml/min) using a variable speed syringe pump (Model 341, Sage Instruments).

Experiment 1

Eight sheep were used to determine the effect of six barbiturates differing in hypnotic activity on feeding behavior. The intraventricular injections were conducted at 2:00 p.m. The drugs tested, the pH of the solutions injected, the drug hypnotic activities [13,16] their partition coefficient [5,13] and the doses injected per animal are presented in Table 1. Synthetic CSF was injected the day previous to the injection of the test drug. Control values were compared against treatment feed intake values using paired *t*-test. Eight observations were obtained for each barbiturate and its control.

Treatments and animals were assigned to a completely randomized experimental design. The treatments studied were: 0.9% NaCl control; pentobarbital: 15, 30, 60 and 120 μmoles/animal; barbital: 60, 120, 240 and 480 μmoles/animal. Feed intake and intraperitoneal temperature were recorded and tested statistically by analysis of variance. Specific treatment comparisons were conducted by the least significant differences test [23].

Experiment 3

Seven sheep were used to study the effect of ultrashort (thiamylal) and short acting (pentobarbital) barbiturates on feeding behavior of sheep. Two doses of each barbiturate were studied: 43 and 97 μmoles/animal. Injections of isotonic saline were used as a control. Feed intake values were compared by *t*-test.

RESULTS

Experiment 1

Average feed intake values for each of the barbiturates tested during various intervals postinjection, are presented in Table 2. During the control, sheep ate about 9% (107 ± 8 g) of their 24-hr feed consumption during the 2 hr period postinjection. Barbiturate injections resulted in different

TABLE 1

PARTITION COEFFICIENTS, PH OF THE SOLUTIONS, LENGTH OF HYPNOTIC ACTIVITY AND DOSE LEVEL OF BARBITURATES INJECTED INTO THE THIRD CEREBRAL VENTRICLE OF SHEEP IN EXPERIMENT 1

Drug	Partition Coefficient	pH of the solution as injected	Length of Hypnotic Activity	Dose/Animal (μmoles)
Barbital	4.5	9.7	Long	100
Phenobarbital	26.3	9.6	Long	40
Amobarbital	89.5	9.6	Intermediate	50
Pentobarbital	89.5	9.7	Short intermediate	40
Secobarbital	143.0	9.5	Short	40
Thiamylal	2,000 approx	9.8	Ultrafast	40

Experiment 2

Six sheep were used to determine their feeding response to various doses of a long acting barbiturate (barbital) and a short acting one (pentobarbital). During surgery, in addition to the third ventricular guide, a piece of silastic tubing sealed in one end was introduced into the peritoneal cavity of the animals (in proximity to the liver) and secured in place by a Dacron mesh skirt cemented to the tubing and sewn under the skin. A temperature probe was introduced through the open end of the tubing to record temperature changes in the peritoneal cavity.

responses with respect to the total feed consumed, but not with respect to the latency of the response. When the short (secobarbital) and ultrashort acting (thiamylal) barbiturates were injected, a 17 and 77 g increase in feed intake above control values was observed at 15 min postinjection. When the intermediate- (pentobarbital, amobarbital) or long acting (phenobarbital, barbital) barbiturates were injected, an increase in feed intake occurred ranging from 117 to 211 g above control values during the 15 min postinjection period (Table 2, $p < 0.01$).

TABLE 2

FEED INTAKE OF SHEEP AS AFFECTED BY THIRD VENTRICULAR INJECTIONS OF VARIOUS BARBITURATES (EXPERIMENT 1)

Treatments	n	Feed Intake (g±S.E.M.) at Different Intervals Postinjection (min)				
		0 – 15	0 – 30	0 – 60	0 – 120	24-hr
Control	8	24±3	40±4	60±5	107±8	1176±59
Barbital	8	229±71*	354±103†	453±110†	484±109†	1543±135*
Phenobarbital	8	179±47†	251±55†	293±52†	336±53†	1311±88
Amobarbital	8	141±32*	180±40*	241±36†	301±35†	1051±132
Pentobarbital	8	235±53†	314±67†	335±65†	351±58†	928±152
Secobarbital	8	41±14	90±27	109±30	156±34	1132±127
Thiamylal	8	101±26*	138±40*	149±44	183±42	1419±142

*Statistically different from control, $p<0.05$
†Statistically different from control, $p<0.01$

Long acting barbiturates elicited consistent and sustained feeding for as long as 2 hr after the injection ($p<0.01$), while short acting barbiturates produced a slight response in some animals and no response in others. The long acting barbital produced an increase in daily feed consumption of approximately 30% above control values (1543 vs 1176 g, $p< 0.05$) during the 24 hr following injections. Injections of pentobarbital, which is considered as short-intermediate, resulted in 24-hr feed intakes lower than that following control injections (928 vs. 1176 g, $p<0.10$).

Experiment 2

Average feed intake values for the different treatments are presented in Table 3. An increase in feed intake was observed in all treatments, at all times postinjection, when compared with control values. The most effective doses to elicit feeding were 240 μmoles for barbital and 30 μmoles for pentobarbital. Thus, to elicit a maximum feeding response, smaller doses of pentobarbital were required than those of barbital. However, the quantity of feed consumed after injections of 240 μmoles of barbital was 309 g larger than after injections of 30 μmoles of pentobarbital during the 1 hr postinjection period ($p<0.01$).

Comparing the feeding responses elicited by equimolar doses (60 and 120 μmoles/animal) of barbital and pentobarbital at 15, 30, 60 and 120 min postinjection showed that barbital elicited larger feeding than pentobarbital at both dose levels. None of the treatments affected 24-hr feed intakes.

Intraperitoneal (IP) temperature was not affected by barbital injections. However, a significant increase in temperature (0.2–0.5°C) was observed in all animals shortly after the injections of pentobarbital at all doses tested (Fig. 1, $p<0.01$).

Experiment 3

As shown in Table 4, the ultrashort acting barbiturate, thiamylal, did not elicit feeding at either of the two doses injected. Pentobarbital injections, on the other hand, resulted in a significant increase in feed intake at both doses. The larger increase was obtained after the injections of 43 μmoles/animal (415 vs. 13 g for pentobarbital and control respectively, at 30 min postinjection, $p<0.01$). When 97 μmoles of thiamylal were injected, the animals showed apparent signs of ataxia. Some animals were unable to remain standing, showing lack of coordination of movements in their attempts to stand up. Others were standing up, but bleating, stamping their feet and rubbing their heads against the cages. All these are signs of discomfort in sheep. Such behavior lasted for a few hours postinjection.

DISCUSSION

The feeding responses observed in sheep after injections of different barbiturates into the third ventricle appeared to be related to the length of hypnotic activity and lipid partition coefficients of the drugs. Long acting barbiturates consistently elicited feeding in all animals injected. At the end of 2 hr, sheep receiving 100 μmoles of barbital had eaten 40% of their normal 24-hr feed intake as compared with control tests when animals consumed in 2 hr only 9% of their daily intake.

Pentobarbital elicited a maximum feeding response at a dose level 8 times smaller than the dose of barbital required for maximum feeding; but, at the maximum effective doses, barbital elicited greater intakes and more sustained feeding than pentobarbital. Short acting barbiturates did not elicit consistent feeding responses.

TABLE 3

FEED INTAKE OF SHEEP AS AFFECTED BY THIRD VENTRICULAR INJECTIONS OF PENTOBARBITAL AND BARBITAL*

Treatments		n	Postinjection Time (min) 0 – 15	0 – 30	0 – 60	0 – 120
Control		6	10 ± 8^{a}	30 ± 6^{a}	31 ± 28^{a}	55 ± 28^{a}
Pentobarbital	15 μmoles	6	135 ± 46^{ab}	145 ± 50^{ab}	146 ± 50^{ab}	147 ± 50^{ab}
	30 μmoles	6	283 ± 130^{bc}	389 ± 179^{cd}	390 ± 179^{bc}	394 ± 178^{bc}
	60 μmoles	6	193 ± 20^{ab}	222 ± 19^{abc}	247 ± 33^{abc}	249 ± 32^{abc}
	120 μmoles	6	205 ± 74^{abc}	282 ± 76^{bc}	290 ± 78^{abc}	293 ± 78^{abc}
Barbital	60 μmoles	6	249 ± 44^{bc}	322 ± 61^{bc}	348 ± 59^{bc}	349 ± 60^{bc}
	120 μmoles	6	237 ± 68^{bc}	343 ± 80^{bcd}	421 ± 86^{cd}	427 ± 85^{cd}
	240 μmoles	6	405 ± 128^{c}	574 ± 150^{d}	699 ± 170^{d}	753 ± 184^{d}
	480 μmoles	6	321 ± 101^{bc}	387 ± 106^{cd}	451 ± 127^{cd}	498 ± 133^{cd}

*Feed intake expressed as means in g ± standard error
[abcd]Means sharing a common superscript within a postinjection time are not statistically different as determined by ANOV and lsd test ($p<0.05$)

TABLE 4

FEED INTAKE OF SHEEP AS AFFECTED BY THIRD VENTRICULAR INJECTIONS OF PENTOBARBITAL AND THIAMYLAL*

Treatments	n	Postinjection Time (min) 0 – 15	0 – 30	0 – 60	0 – 120
Control	6	8 ± 2	13 ± 4	25 ± 5	38 ± 8
Pentobarbital					
43 μmoles	4	303 ± 77‡	415 ± 97‡	420 ± 97‡	420 ± 97‡
97 μmoles	6	186 ± 36†	223 ± 49†	223 ± 49†	224 ± 49*
Thiamylal					
43 μmoles	4	10 ± 4	26 ± 15	30 ± 17	62 ± 34
97 μmoles	3	21 ± 11	36 ± 20	40 ± 22	87 ± 54

*Feed intake expressed as means in g ± standard error
†Statistically different from control, $p<0.05$
‡Statistically different from control, $p<0.01$

In spite of the similar dose levels and anesthetic properties, pentobarbital, secobarbital and thiamylal produced different responses. At various doses injected, pentobarbital elicited marked feeding within 2 min after the injections terminated, while secobarbital and thiamylal elicited only short lasting and variable responses. Such differences could be explained in terms of the lipid solubilities of barbiturates. Secobarbital and thiamylal have very high solubilities being capable of entering and leaving biological membranes very rapidly, resulting in only a short length of action [4,14]. They may diffuse away from areas responsible for the feeding observed or be metabolized by brain tissue. There is evidence that sulfur containing barbiturates (ultrashort acting, e.g. thiopental, thiamylal) are degraded in brain tissue while long acting barbiturates are not [7]. This could explain why there was such a small

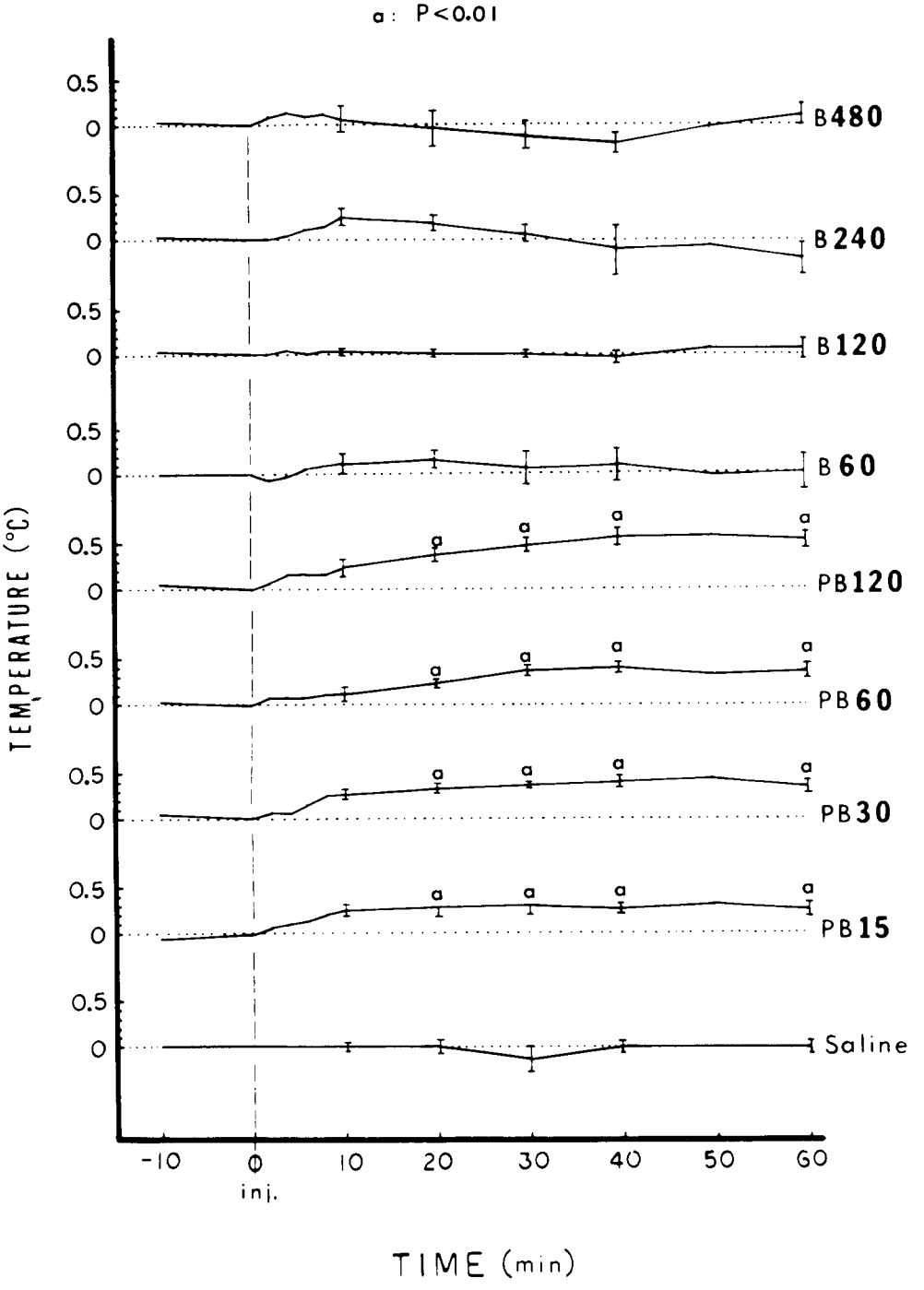

FIG. 1. Effects of various doses (μmoles) of barbital (B) and pentobarbital (PB) on intraperitoneal temperature of sheep in Experiment 2. IP temperature is expressed as changes in centigrade degrees from the temperature observed at the time of injection (0°C).

response after thiamylal injections.

No differences were observed between effective barbiturates in relation to the latency of the feeding response. All barbiturates, at doses that elicited feeding, did so within 2 min after the injections terminated with slight variations between animals. Thus, the latency of the response was not a determinant of the magnitude of feeding elicited.

The least lipid soluble of the barbiturates injected, barbital, elicited the greatest feeding response. At the end of 1 hr, animals receving barbital continued to eat small quantities of feed, while animals receiving pentobarbital were lying down with no signs of hunger. These observations could be related to the length of hypnotic action of the drugs. Barbital is the simplest derivative of barbituric acid possessing hypnotic properties. It is regarded as a poor

hypnotic and anesthetic because of its low partition coefficient, which decreases greatly its lipid solubility [4,13]. Consequently it penetrates membranes at a very slow rate. This is reflected in its slow onset of action. As the nature of the substituents in the 5-carbon position of the barbiturate molecule are made more lipophilic, or with the introduction of a sulphur group in the 2-carbon position, the solubility of the barbiturate increases [16]. Although there is not direct evidence from our experiments, the solubility properties of barbital suggest that this drug exerted its feeding response by acting in the proximity of the site of injection. Since barbital penetrates membranes very slowly, by the time the feeding response was observed (2 min postinjection), it probably had not penetrated more than the periventricular tissue within the region of the third ventricle. The 24-hr increase in feed intake observed after barbital injections suggest that this compound may remain longer at the site of action without undergoing appreciable metabolism.

The increase in body temperature observed shortly after pentobarbital injections tends to support the previous assumptions. Pentobarbital, but not barbital, appears to have diffused into hypothalamic areas involved in temperature regulation [17]. The low solubility of barbital may have been responsible for its lack of effect, preventing it from reaching brain structures located not adjacent to the third ventricle. The data also suggest that the feeding effect was independent of temperature regulation, since feeding was elicited by barbital without affecting body temperature. All doses of pentobarbital elicited similar increases in body temperature, however, the feeding responses were markedly different.

Although other regions in the limbic system can not be excluded, we suggest that barbiturates elicited feeding in sheep by acting either on the ventricular membrane itself or on the ventromedial area of the hypothalamus. The role of the VMH in feeding behavior is widely recognized [24]. Since its location is within 1.5 mm at either side of the third ventricle and the latency of the feeding response was similar for all effective barbiturates injected, we assume that the VMH may be the site of action of these drugs.

Previous investigations have shown that pentobarbital injections into the lateral ventricles of hyperphagic rats induced feeding in spite of lesions in the ventromedial area of the hypothalamus [15]. Baile *et al.* [2] recently demonstrated that feeding can be induced in satiated sheep by injections of local anesthetics into the medial hypothalamus. Increased feeding may also result from lesioning other areas adjacent to the VMH [9] and by suppressing neural information going to the lateral hypothalamus [11,12]. Barbiturates probably exert their feeding response in a similar way; that is, they may depress neural activity of inhibitory fibers acting on the feeding center of the lateral hypothalamus, thus eliciting feeding.

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