some LDH_4. This excludes plasma, dermis, platelets, leucocytes, and erythrocytes as the source of the enzyme. At 12 hours, appreciable amounts of LDH_1, LDH_2 and LDH_3 are present in blister fluid, which could be derived from leucocytes or platelets. This is consistent with the observations of Smith *et al* (16) who found few leucocytes present in blister fluid 7 hours after the application of cantharidin, but found many cells 5 hours later.

Summary. Lactate dehydrogenase (LDH) isozyme patterns in the blister fluid of cantharidin-produced blisters, epidermis, dermis, leucocytes, platelets, and serum have been studied. Blister fluid collected 7 hours after cantharidin application contained mainly LDH_5 with some LDH_4. LDH_2 and LDH_3 also appeared in blister fluid collected 12 hours or longer after application of cantharidin. This finding is consistent with the hypothesis that LDH in cantharidin-produced blisters is derived initially from epidermis and is later augmented from leucocytes or platelets.

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Pregnanolone: A Highly Potent, Naturally Occurring Hypnotic-Anesthetic Agent. (32276)

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The first findings of Selye in 1940(1) on the hypnotic effect of certain hormonal steroids have since been substantially expanded. A group of water soluble ester salts of 5 beta-pregnane derivatives with hypnotic effects and lacking hormonal activities were described 10 years ago(2). One of these compounds, hydroxydione, the sodium hemisuccinate salt of 21-hydroxy pregnanedione has been introduced as an intravenous anesthetic agent. Some side effects, however, limited its therapeutic usefulness. Recent efforts to obtain suitable and more potent water soluble anesthetic steroids did not meet with much success(3,4).

Of the hormonal steroids studied by Selye (1) progesterone showed considerable potency. Since it was subsequently shown that some of the naturally occurring metabolic products of this hormone are even more potent hypnotics (2) a neuropharmacologic exploration comparing progesterone with these hormonally inactive but hypnotically potent metabolites has been made(5). It was found that a) the free

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alcohol or ketone forms of pregnanes related to progesterone, when administered intravenously in non-aqueous solvents to experimental animals were more potent than either their water soluble ester salt forms or the water suspensions of the corresponding free alcohols and ketones used previously, and b) a metabolite of progesterone: 3 α-hydroxy 5 β-pregnane -20- one (pregnanolone) exhibited outstanding hypnotic potency. The above findings indicated a more detailed neuropharmacologic study with this agent.

Materials. Pregnanolone (3α-hydroxy 5-β-pregnane 20-one) (Mann Research Labs., New York, Lot #522/M1200), Thiopental sodium (Abbott), Pentobarbital sodium (Abbott), Phenobarbital U.S.P., Hydroxydione hemisuccinate (Pfizer).

Methods. 1. a) i.v. and i.p. administration of the drugs in propylene glycol (pregnanolone) or saline to male Swiss albino mice to determine the median hypnotic and lethal doses; (ED50 for loss of righting reflex and LD50); b) i.v. administration of pregnanolone to rats, cats, rabbits, dogs, and 4 species of monkeys to determine minimal hypnotic doses.

2. Behavioral experiments on rats with a Sidman avoidance schedule, with i.p. administration. Male Long Evans rats were subjected in Skinner boxes to a continuous avoidance behavior schedule with a RS interval of 40 seconds and an SS interval of 20 seconds. Continuous recordings were made for 4-hour sessions.

3. Electroencephalographic study with chronic, unanesthetized cats and squirrel monkeys carrying implanted cortical and deep electrodes. The implanted cats were freely moving in a shielded cage in an isolation box. The EEG and motor behavioral responses of the arousal reactions elicited from stimulating the midbrain reticular formation (RF) and/or hypothalamus, thalamus and internal capsule, and the localized or generalized seizure discharges following hippocampal stimulation were recorded and evaluated before and after drug administration.

4. Acute cat and monkey experiments with gallamine immobilized animals, recording: a) electrocortical activity; b) evoked single potentials in the brain stem using intravenous administration.

5. Tests on anesthetized cats recording blood pressure, respiration, nictitating membrane function and intestinal motility.

TABLE I. Hypnotic and Lethal Doses of Pregnanolone, Hydroxydione and Thiopental in Mice.

Compound	Median hypnotic dose* (ED50), mg/kg i.v.	i.p.	LD50, mg/kg i.v.	Ratio LD50/ED50
Pregnanolone	2.3 ± .33	20 (400)	66 ± 10	29
Hydroxydione	39 [22]	40	160	4
Thiopental	18.5 [17.5]		84 [80]	4.4

* Loss of righting reflex.

No. of animals for pregnanolone varied between 27 and 40 for each test. Approximate values for hydroxydione and thiopental were obtained on 15-30 animals each.

Values in parentheses refer to oral administration.

Values in brackets were reported by P'An *et al* (1955).

Results. Pregnanolone was administered throughout these experiments in propylene glycol solution of various concentrations. Comparison of the hypnotic and lethal doses of pregnanolone in mice as related to another steroidal and hypnotic agent (Hydroxydione) is shown in Table I. It should be noted that a) pregnanolone is much less potent given i.p. than i.v. (it was almost inactive when given orally), b) the ratio between the lethal and hypnotic dose is very favorable. Since the ED 50 and LD 50 of pregnanolone were 2.3 and 66 mg/kg and of Thiopental were 18.5 and 84 mg/kg, respectively, the relative hypnotic potency of pregnanolone is 8 times that of thiopental while its acute toxicity is only slightly (about 1.3 times) more pronounced. Earlier reports with pregnanolone and related 5 β-pregnanes indicated considerably lower potency, for the reason that the compounds were either given as water suspensions or in the form of their water soluble ester salts.

The hypnotic effect of pregnanolone was further determined on 8 different mammals including 4 species of monkeys. Uniform results were obtained regarding potency and duration of action (Table II). The minimal effective doses of pregnanolone are 10-20

TABLE II. Hypnotic Doses and Duration of Sleep in Different Mammals (Doses in mg/kg i.v., Solvent: Propylene Glycol).

Species	Pregnanolone	Hydroxydione	Thiopental	Pentobarbital
Rat	2.5 (5–10)	26*		
Rabbit	1.2 (14–15)	15*	10 (6)	
Cat	1-2 (6–15)			
Dog	2-4 (10–15)	50*		
Squirrel monkey *(Saimiri sciurea)*	1.8 (7– 8)	33 (25–30)	7 (8–10)	9 (30)
Capuchin monkey *(Cebus apella)*	2.4 (3– 4)	54 (30)	7 (8–10)	12 (17)
Pigtail macaque *(M. nemestrina)*	1.6 (2– 4)	30 (20)	5 (5)	10 (10)
Stamptail macaque *(M. speciosa)*	1.8 (9)			12 (15)

* See reference(2). Numbers in parentheses: duration of sleep in min.

times lower than that of hydroxydione and 3-8 times lower than that of the barbiturates used. In contrast to hydroxydione with pregnanolone there is immediate sleep and loss of reflexes. At the minimal effective dose level the duration of action of pregnanolone is considerably shorter than with hydroxydione and pentobarbital and even somewhat more fleeting than with thiopental. Awakening is extremely fast and with the exception of cats and squirrel monkeys, which exhibit slightly increased reflex excitability and minor muscular twitching, the recovery is symptom-free without ataxia, commonly observed even with the ultrashort acting barbiturates on experimental animals.

The minimal effective doses (average of 4 animals) suppressing avoidance behavior on rats following i.p. administration were: pregnanolone 27 mg (10-40 mg/kg), hydroxydione 40 mg/kg. Phenobarbital was effective at 30 mg/kg in 3 out of the 4 animals tested. Doses which inhibited avoidance influenced escape reaction to a lesser extent. The behavioral observations in rats after intraperitoneal administration indicate that pregnanolone by this route is only as effective as the reference hypnotic agents used. This finding parallels those obtained in mice showing relatively inferior potency of this agent when given by the i.p. route (Table I).

In chronically electrode-implanted cats with i.p. administration pregnanolone produced sleep spindles of the EEG at 10-20 mg/kg and suppressed the cortical arousal reactions and hippocampal seizures at 40 mg/kg. Hydroxydione was effective in the same dose range, while pentobarbital was about twice as potent as the 2 steroidal hypnotic agents. The majority of the experiments were using the i.v. route of administration. The results were grouped into 3 categories: 1) Changes in spontaneous electrocortical activity; 2) Behavioral and EEG changes of the reticular arousal threshold; 3) Changes in the rhinencephalic seizure threshold. Table 3 indicates that pregnanolone was markedly more potent than either hydroxydione or pentobarbital sodium.

High voltage spindles of the electrocortical activity, the first signs of behavioral drowsiness and sedation, appeared at .12 mg/kg in the electrode implanted cats, which corresponded to the findings obtained in intact animals producing hypnosis in low doses. Doses of 1-2 mg antagonized the cortical arousal reactions following stimulation of the RF, other deep brain structures and rhinencephalic seizure discharges following hippocampal stimulation. Hydroxydione and pentobarbital were much less effective (Table III). The doses of pregnanolone mentioned above were effective in blocking cortical arousal and rhinencephalic seizure reactions in the monkey (Fig. 1).

In a few acute cat and monkey *(Cebus apella)* experiments, results similar to those observed in the chronic experiments were obtained. Cortical spindle bursts appeared after 0.12-0.25 mg/kg doses in these gallamine immobilized animals. Blood pressure recordings showed a hypotensive effect of only moderate

TABLE III. Comparison of Effects of Pregnanolone, Hydroxydione and Pentobarbital on Electrocorticogram of Cats with Chronically Implanted Electrodes. Values for minimal effective doses were obtained on 3 animals, each of them tested in repeated trials with all 3 compounds.

Compound	Minimal effective doses, mg/kg			
	Sleep spindles	Producing suppression of arousal from the RF	Suppression of rhinencephalic seizures	Notes
Pregnanolone	.12	1–2	1–2	Immediate action
Hydroxydione	5	20	20	Latency of 2-3 min
Pentobarbital Na	3	8	4	Immediate action, deepening at 5-10 min

degree and lasting for 5-10 minutes following the doses of 0.5-2 mg/kg which were sufficient to block cortical arousal reactions. Four mg/kg produced definite suppression of electrocortical activity and also significant BP fall (40-60 mm).

Earlier neuropharmacologic studies indicated many similarities in the action of hydroxydione and intravenous barbiturates (6,7), and suppression not only of ascending but also descending activating pathways passing through the midbrain reticular formation has been implicated in the action of these agents(7,8). In 4 acute cat experiments the responses to pregnanolone and hydroxydione on the evoked potentials in the RF were studied in a similar manner as has been described for pentobarbital elsewhere(8). The potentials elicited by paired electric shocks of 2-12 v, 1 m.sec delay and 1 m.sec duration, separated usually by 200 m.sec. and evoked every 10 seconds, were recorded from different parts of the RF. Stimulating electrodes were placed at the sciatic nerve, at the RF, at the internal capsule or at different thalamic nuclei. A moderate depression of the ascending and descending pathways, reaching the RF occurred at 0.5 mg/kg of pregnanolone and 20 mg/kg of hydroxydione while 2 and resp. 40-80 mg/kg were required to produce marked depression of either pathway (Fig. 2).

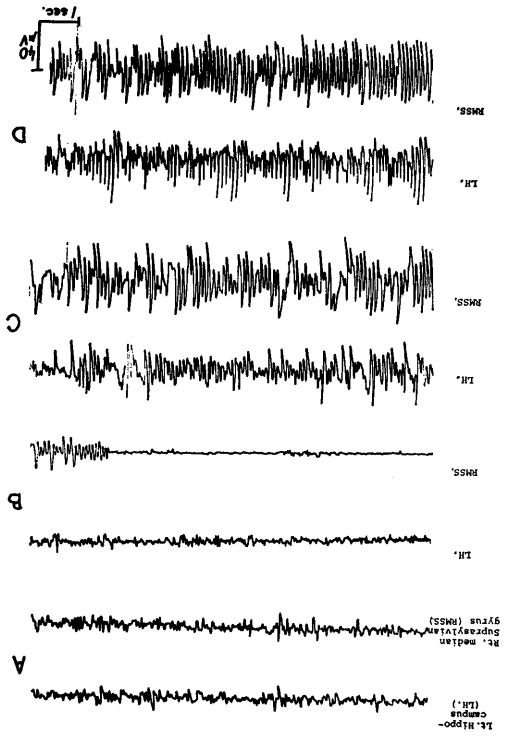

FIG. 1. Effect of pregnanolone on electrocorticogram of the squirrel monkey. A = Before drug, B = Before drug following RF stimulation of 1.5V., cps, C = 2 min after 2 mg/kg pregnanolone, D = Same as C following RF stimulation of 4.5V., 100 cps.

Observations on the respiratory, cardiovascular, and other autonomic functions in cats under pentobarbital anesthesia revealed that fairly small additional anesthetic doses of pregnanolone (0.5-2.0 mg/kg) diminished respiratory volume and rate, produced hypotensive effect, and inhibited intestinal motility for 1-3 minutes, but no suppression of ganglionic transmission and no influence on the pressor response to epinephrine occurred up to 6 mg/kg doses. Comparison with thiopental showed that in the equipotent hypnotic dose the latter exhibited higher degree of respiratory, cardiovascular, and ganglionic depression.

Discussion. Although it was recognized earlier that pregnanolone is a naturally occurring agent and a metabolite of progesterone, the relationship between the actions of

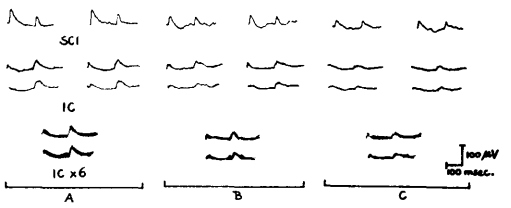

FIG. 2. Effect of 3α-hydroxy 5β-pregnane, 20-one (P) on evoked potentials in the reticular formation. (RF). SCI = paired single shocks of 200 msec. interval, 2 V and 1 msec. duration delivered to the sciatic nerve in each 10 sec. leading to the RF. IC = similar paired shocks of 10 V delivered to the ipsilateral internal capsule leading to the RF. IC 6× = same as IC but 6 superimposed potentials evoked with a frequency of 3/sec. A = Control B = 2-4′ after 0.5 mg/kg. P. C. = 2-4′ after 2 mg/kg. P.

progesterone on the CNS in relation to its metabolic transformation products were not explored until recently. The possibility of giving precursors and metabolites of progesterone i.v. in solution form to different species of experimental animals provided a new means of studying the CNS effects of the parent hormonal compound in relation to its metabolites. The results showed that the recognized metabolic chain leading from progesterone through pregnanedione to pregnanolone is reflected in the onset of action of these agents (5,6). Pregnanolone in the present study was found to be a very potent, short-acting hypnotic agent in experimental animals exhibiting a favorable ratio between hypnotic and lethal doses. The neurophysiologic phenomena observed with pregnanolone were similar to those produced by short-acting barbiturates. However, more refined analysis is necessary to clarify whether the mode of action of these chemically different hypnotic agents is indeed identical.

In view of the above findings, it seems feasible to explore experimentally the possibility that the clinically observed sedative and sometimes hypnotic actions of progesterone (*i.e.*, during pregnancy and following large *i.v.* doses to humans) are mediated through pregnanolone(9).

Summary. Pregnanolone (3α-hydroxy 5β-pregnane 20-one) a metabolite of progesterone was found to be a highly potent, short-acting hypnotic agent when administered in solution form intravenously. Neuropharmacologic analysis in cats and monkeys showed depression of the reticular activating system of the midbrain and other electrophysiologic phenomena similar to those produced by barbiturates. On the basis of the doses employed in this study to a wide variety of mammals, pregnanolone is so far considered to be one of the most potent hypnotic-anesthetic agents.

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