ADH-Like Effect of Tranquilizers in Amphibians.* (28085)

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Administration of certain agents depressing the central nervous system, including barbiturates, morphine, codeine, reserpine and chlorpromazine, has been reported to induce antidiuresis(1-5). These reports suggest that decreased urine production is caused by stimulation of antidiuretic hormone (ADH) secretion.

Since the water balance in amphibians responds to neurohypophyseal ADH stimulation with increased water uptake through the skin(6,7), resulting in body weight gain within 1-5 hours, a bioassay of mammalian antidiuretic hormone on *Bufo marinus* was suggested(8).

The purpose of the present study was to find out whether tranquilizers can effect body weight gain in amphibians by stimulating endogenous ADH release.

Materials and methods. I. *Intact animals.* 100 adult male and female toads (*Bufo viridis*) and 100 frogs (*Rana pipiens*), weighing 30-60 g each, were used. Each animal was kept separately in a glass jar in 4 cm high water which was changed every day. Before determination of the individual weight, each animal was made to excrete the accumulated urine by wrapping it in a piece of towel and slight squeezing of the lower abdomen. Groups of 10 frogs were injected in the dorsal lymph sac with 0.2 ml of one of the following preparations: physiological saline (controls), frog neurohypophyseal homogenates (½ pituitary), ADH (1 U Pitressin, Parke-Davis), and 17 different tranquilizers. Body weight was registered every half hour for 6 hours, and also after 24 and 48 hours.

II. *Hypophysectomized animals.* Three groups of 10 hypophysectomized toads were injected with 1 IU ADH (Pitressin), 2.5 mg chlorpromazine (Largactil) and 0.2 ml physiological saline. Body weight was compared with that of 10 intact animals receiving the same treatment.

Results. I. *Intact toads and frogs.* Injection of 0.2 ml physiological saline produced no body weight change. However, the 2 groups treated with homogenates of toad neurohypophysis and with the commercial ADH preparation showed a significant body weight gain. Fig. 1 gives percentages of body weight gains effected by frog posterior pituitary homogenate, ADH, phenobarbitone sodium, promethazine, chlorpromazine, prothipendyl, trifluperazine and reserpine respectively in frogs and toads 6, 24 and 48 hours after injection. Among the tranquilizers, chlorpromazine showed the strongest ADH-like effect (Table I, Fig. 2), while chlordiazepoxide, meprobamate, trifluperazine, benactyzine, pipamazine, and reserpine were less active. Perphenazine, serotonin and methopromazine produced no significant changes. Moreover, the effect of the tranquilizers which induced water uptake and body weight gain, such as promethazine, chlorpromazine and prothipendyl, continued for 48 hours, while ADH lost its effectiveness within 24 hours (Fig. 1). There were no differences in reaction between frogs and toads.

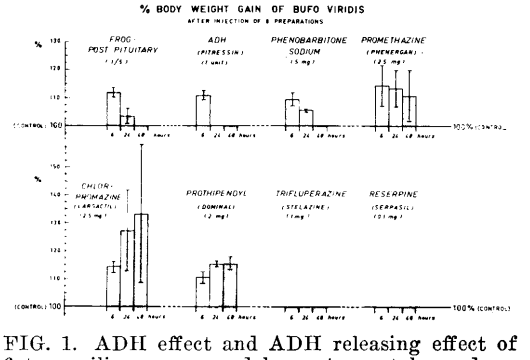

FIG. 1. ADH effect and ADH releasing effect of 6 tranquilizers measured by water uptake and retention in amphibians. Percentages of body wt gains with standard deviations, 6, 24 and 48 hr after injection of above preparations.

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TABLE I. Body Weight Gain of *Bufo viridis* and *Rana pipiens* 6 Hours After s.c. Injection of Different Drugs.

Drug Generic and proprietary name	Dose (mg/animal)	Body wt gain Mean ± S.E.M. (%)
Control (Saline)	.2 ml	2.3 ± .22
Pitressin (P.D.)	1 IU	15.3 ± 1.45
Frog pituitaries	½ Pit.	12.6 ± .26
Chlorpromazine (Largactil)	2.5	20.7 ± 3.10
Prothixene (Taractan)	2.5	15.2 ± 1.76
Phenobarbitone sodium (Luminal)	5.0	11.2 ± 1.26
Prothipendyl (Dominal)	2.0	10.5 ± 1.44
Butyrylperazine (Randolectil)	1.0	9.6 ± 2.20
Triflupromazine (Siquil)	2.5	8.9 ± .17
Hydroxyzine (Atarax)	1.0	8.7 ± 1.04
Promethazine (Phenergan)	2.5	7.3 ± .28
Levomepromazine (Nozinon)	2.5	6.1 ± .86
Chlordiazepoxide (Librium)	1.0	6.0 ± 1.60
Meprobamate (Miltown)	10.0	5.1 ± 1.50
Trifluoperazine (Stelazine)	1.0	4.1 ± .86
Benactyzine (Suavitil)	1.0	3.9 ± .17
Pipamazine (Mornidine)	.5	3.7 ± .23
Reserpine (Serpasil)	.1	3.6 ± .10
Perphenazine (Trilafon)	.5	2.9 ± .10
Serotonin creatinine sulfate	2.0	2.2 ± .15
Methopromazine (Mopazine)	5.0	1.0 ± .63

II. *Hypophysectomized toads* treated with ADH showed a body weight gain similar to that of the intact animals, but chlropromazine treatment gave a much weaker effect in the former (Fig. 3).

Discussion. Endocrine disturbances caused by tranquilizers are presumed to result from their action on the diencephalon, and particularly the hypothalamic region(9). In mammals, interference of tranquilizers with normally-functioning anterior and posterior lobes of the pituitary was reported by us, especially depression of anterior pituitary FSH, LH and STH, but increase of production and secretion of prolactin, MSH, ADH and oxytocin,

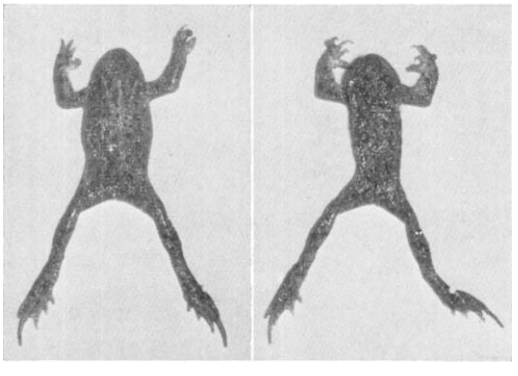


FIG. 2. Two toads. Right—before treatment: normal size. Left—24 hr after injection of 2.5 mg chlorpromazine: 35% gain in body wt by water uptake and retention.

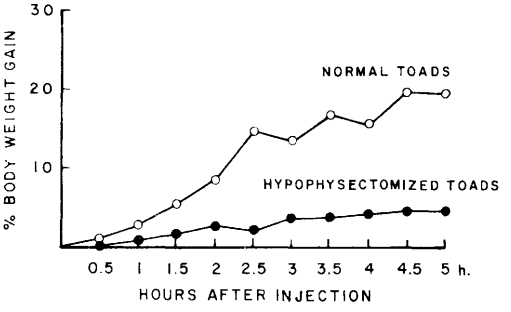
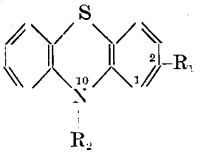


FIG. 3. Avg values of water uptake and retention measured by body wt gain of 10 normal and 10 hypophysectomized toads after injection of 2.5 mg chlorpromazine.

and transient elevation of ACTH secretion. We have explained these complex phenomena by the existence of balanced and unbalanced tropins(10,12-14,16). Balanced tropins produce secondary hormones, unbalanced ones produce a direct organ reaction. In amphibians, phenothiazine derivatives, as well as reserpine, were found to induce MSH release, leading to blackening of the skin(15,16). The effect of neurohypophyseal extracts of ADH on toad and frog skin consists of dilation of minute pores, leading to increased water uptake and consequently to body weight gain (17).

The mammotropic effect of tranquilizers in mammals and activity in toads was stud-

TABLE II. Structure-Activity-Relationship of Phenothiazine Derivatives Assayed for Body Weight Gain in Frogs and Toads.



Phenothiazine drugs generic and proprietary name	Substitution		t-test related to control
	R_1	R_2	
Chlorpromazine (Largactil)	Cl	$-CH_2-CH_2-CH_2-N(CH_3)(CH_3)$	$P < .015$
Prothixene* (Taractan)	H	$-CH=CH-CH_2-CH_2-N(CH_3)(CH_3)$	$P < .015$
Prothipendyl† (Dominal)	H	$-CH_2-CH_2-CH_2-N(CH_3)(CH_3)$	$P < .015$
Butyrylperazine (Randolectil)	$-C(=O)-CH_2CH_2CH_3$	$-CH_2-CH_2-CH_2-N$ (piperazine) $-N-CH_3$	$P < .04$
Triflupromazine (Siquil)	CF_3	$-CH_2-CH_2-CH_2-N(CH_3)(CH_3)$	$P < .01$
Promethazine (Phenergan)	H	$-CH_2-CH(CH_3)-N(CH_3)(CH_3)$	$P < .015$
Levomepromazine (Nozinan)	OCH_3	$-CH_2-CH(CH_3)-CH_2-N(CH_3)(CH_3)$	$P < .015$
Trifluoperazine (Stelazine)	CF_3	$-CH_2-CH_2-CH_2-N$ (piperazine) $-N-CH_3$	$P < .05$
Pipamazine (Mornidine)	Cl	$-CH_2-CH_2-CH_2-N$ (piperazine) $-C(=O)-NH_2$	$P > .01$
Perphenazine (Trilafon)	Cl	$-CH_2-CH_2-CH_2-N$ (piperazine) $-N-CH_2-CH_2-OH$	$P < .05$
Methopromazine (Mopazine)	OCH_3	$-CH_2-CH_2-CH_2-N(CH_3)(CH_3)$	$P > .1$
Control (saline)			—

* The N atom at position 10 is substituted by C.
† " C " " " " 1 " " " N.

ied with regard to their structure-activity-relationship(14,15). The phenothiazine tranquilizers (Table II) give an ADH-like effect by inducing increased water uptake through the skin of frogs and toads. This leads to maximum weight gain when R_1 at position 2 is Cl and R_2 at position 10 is a dimethyl amino-propyl group, as *e.g.*, chlorpromazine (Largactil). Substitution of the Cl atom at position 2 by H, as in prothixene (Taractan), prothipendyl (Dominal), promethazine (Phenergan), or by CF_3 as in Triflupromazine (Siquil), trifluoperazine (Stelazine), or by OCH_3 as in levomepromazine (Nozinan), methopromazine (Mopazine), results in a weaker ADH-like effect. A branched R_2 chain at position 10 or the substitution of the dimethylaminopropyl group by a piperazinepropyl group, as in butyrylperazine (Randolectil), trifluoperazine (Stelazine), pipamazine (Mornidine), perphenazine (Trilafon), reduced the ADH-like effect of increased water uptake. Other tranquilizers, not belonging to the phenothiazine group, such as chlordiazepoxide, meprobamate, benactyzine, reserpine were less active.

It is noteworthy that the clinical value and ranking of phenothiazine derivatives in treatment of psychotic patients is reported to be parallel to their MSH and LTH releasing effect(18). Our results show that it is equally parallel to their ADH-releasing activity.

Chlorpromazine was the most effective drug in inducing body weight gain in intact frogs and toads, but was only slightly active in hypophysectomized animals. This would indicate that some endogenous ADH secretion was produced by action on the hypothalamus, but the main effect, ADH release from the posterior pituitary, could not take place.

Reserpine, on the contrary, produced only little body weight gain. This finding agrees with our report(5,19) that reserpine antidiuresis in water loaded rats and dogs seems to be due to serotonin depletion rather than to ADH hypersecretion.

Oxytocin has been reported to prevent the ADH effect and water uptake in toads(20). Yet our results with toads and frogs (Fig. 1) were not noticeably influenced by this antagonism.

Summary. Seventeen tranquilizers were studied for their ADH-releasing effect of inducing increased water uptake and body weight gain in *Bufo viridis* and *Rana pipiens*. In intact amphibians phenothiazine derivatives produced a varying degree of increased water uptake through the skin. Chlorpromazine was found to be even more effective than either exogenous ADH (pitressin) or pituitary homogenates since its effect lasted for 24-48 hours. Phenothiazine derivatives with a Cl-atom at position 2 and a dimethylaminopropyl side-chain at position 10 gave the maximum effect. On the other hand, substitution of Cl at position 2 by H, CF_3 or OCH_3, or of the dimethylamino-propyl group by a branched chain, or a piperazinepropyl group, reduced the ADH-like effect. Other tranquilizers, such as chlordiazepoxide (Librium), meprobamate, benactyzine, reserpine showed no significant effect. Treatment of hypophysectomized toads and frogs with chlorpromazine produced a very weak effect, apparently due to ADH release from the hypothalamus only. On the basis of the above findings it is suggested that through the action of tranquilizers on the hypothalamus, ADH hypersecretion from the hypothalamus and release from the posterior pituitary can be effected, resulting in an increased water uptake and body weight gain in Bufo toads and Rana frogs.

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A Technic for Separation of the Cells of the Gastric Mucosa.* (28086)

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Cellular biochemical and metabolic activity is most suitably investigated in a preparation of a single cell type. A suspension of living parietal cells, although frequently discussed by those interested in gastric physiology, has not been available(1). This communication reports a technic that provides a suspension of living parietal cells. Although not pure, it is a good working model with which to pursue further purification of the preparation as well as to study the physical and chemical activities of this unique cell.

Material and method. Gastric mucosa from young rabbits is used because of the facility with which it lends itself to this technic, although the procedure yields almost equally good results with dog and human gastric mucosa. The rabbit is sacrificed with sodium pentobarbital, the gastric fundus immedately removed, rinsed in a salt solution (GKN) and the mucosa manually stripped from submucosa and muscularis. The tissue is then minced with scissors, rinsed again with the salt solution and placed in an extraction flask for digestion in collagenase for 2 hours at 37°C. After rinsing, the suspension is strained through an ordinary blood-plasma administration set filter, rinsed several times and layered on gradient density media for differential centrifugation. The gradient is a long chain sucrose polymer (*FICOLL, Pharmacia Laboratories) prepared in advance by layering upon one another solutions of FICOLL of specific gravity 1.07, 1.05 and 1.02 respectively. The preparation is centrifuged for 12 minutes at 1000 rpm, the cells of each layer removed and rinsed. Smears of the various fractions are made by transferring material to a slide with a pipette, air drying and fixing in appropriate solutions for staining.

Cell survival is determined by trypan blue staining. Tetrazolium salts are used to stain for succinic dehydrogenase using the method

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