Role of Pituitary in Cold-Induced Brain Hyperexcitability.* (28991)

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Exposure to moderate cold (2°C) induces brain hyperexcitability in adult male rats, as indicated by a decrease in electroshock seizure threshold(1). The increased susceptibility to seizures is accompanied by adrenal enlargement, thymic involution, and alterations in electrolyte and carbohydrate metabolism in brain, liver, muscle, and plasma(1). These changes indicate that pituitary and adrenal cortical hormones may be involved in this response.

To determine whether endocrine factors related to the hypophyseal-adrenocortical axis are responsible for the observed changes in brain excitability, hypophysectomized rats were placed in the cold, and changes in their electroshock seizure threshold were determined and compared with those of intact control rats.

Methods. Animals. Sixteen male adult hypophysectomized Long-Evans rats were fed a standard powder diet and drank a 5% glucose solution, both *ad libitum*. Twelve non-operated control rats of the same age, sex and strain received the same diet.

Cold exposure consisted of placing the animals in a room at a constant temperature of 2°C and humidity of 66%, in individual cages with wire bottoms, thereby standardizing the amount of cold stress on each animal. Hypophysectomized rats weighing an average of 170 g were placed in the cold one month after the operation, together with non-operated controls of the same age and weighing an average of 195 g.

Seizure threshold was determined with the apparatus and techniques described in detail by Woodbury and Davenport(2). Convulsions were elicited with a stimulator which delivers 60 cycles/sec of sinusoidal current through corneal electrodes for a fixed period of time. The stimulator has a constant-current circuit with a high voltage, in series with a high resistance and with the animal, the stimulus therefore being independent of variations in the resistance of the animal. The electroshock seizure threshold is defined as the minimum amount of current, in milliamperes (ma), delivered through corneal electrodes for 0.2 sec, which results in a barely detectable clonic convulsion of the ears, jaws, vibrissae, and forepaws, lasting only a few seconds and without loss of equilibrium.

Seizure threshold determinations were performed twice weekly on all rats for 3 weeks preceding cold exposure to familiarize the animals with handling and procedure and to determine pre-exposure threshold values accurately. During cold exposure, electroshock determinations were made directly in the cold room.

Body weights were determined before each electroshock test. *Statistics* were determined by means of Student's "t" test.

Results. A first group of 8 hypophysectomized and 6 normal rats was placed in the cold in the evening, with food and drink *ad libitum*. After 6 hours in the cold, the seizure threshold had dropped slightly in the 6 controls (—0.9%) and markedly in the 4 surviving hypophysectomized rats (—5.7%) (Table I). Body weights also differed markedly, with the hypophysectomized rats losing about as much weight as the controls had gained over the 6-hour period of cold exposure. Examination of food containers showed that while the control rats had eaten during the night, as expected, the hypophysectomized rats had not.

To isolate the threshold-lowering effects of cold exposure from those of fasting, the experiment was repeated on another group of 8 hypophysectomized and 6 control rats. Both groups of animals were placed in the cold in the morning, after a presumably nutritious night, with drink *ad libitum,* but without food. Seizure thresholds and body weights were determined after 3 hours in the cold

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TABLE I. Electroshock Seizure Threshold and Body Weight of Hypophysectomized and Intact Adult Male Rats After Exposure to Moderate Cold.

Food	Parameter determined	Cold exposure duration (hr)	Control (% change)*	Hypophysectomized (% change)*	P†
Ad libitum	Body wt	6	+3.8 ± .2‡	−4.6 ± .6	≤.001
	Electroshock threshold	6	− .9 ± .6	−5.7 ± 1.6	≤.05
Removed	Body wt	3	−5.4 ± .9	−4.6 ± .4	
		6	−6.1 ± 1.0	−5.9 ± .3	
	Electroshock threshold	3	− .5 ± .5	−2.9 ± .5	≤.01
		6	−1.6 ± .4	−5.9 ± 1.0	≤.02

* From pre-exposure values.
† For differences between control and hypophysectomized rats.
‡ Mean ± S.E.

(1 hypophysectomized rat dead) as well as after 6 hours (3 hypophysectomized rats dead). The difference in seizure threshold between control and hypophysectomized rats was as pronounced as in the previous experiment, despite identical changes in body weight (Table I).

Discussion. Our results show that the increased brain excitability observed in normal rats upon exposure to moderate cold cannot be related to pituitary hyperfunction. Indeed, the fall in seizure threshold is much greater in hypophysectomized than in intact rats exposed to cold.

Thyroid and glucocorticoid hormones are known to be secreted in greater amounts in the cold(3), and to lower seizure threshold(4, 5). In the present investigation, however, hypophysectomy further increased rather than diminished brain hyperexcitability of cold-exposed rats, contrary to an expected decreased convulsability in the absence of thyroid and glucocorticoid responses.

The increased excitability of cold-exposed hypophysectomized rats could be attributed at least in part to the absence of mineralocorticoid secretion in these animals. Mineralocorticoids are known to decrease brain excitability(5). Their secretion is normally increased in the cold due to the action of renal pressor substances(6) which are secreted by the kidney in response to cold-induced nephrosclerosis(7) and constriction of the renal vessels(8). Further evidence for an increased mineralocorticoid secretion in the cold is the accompanying sodium retention and potassium depletion(1). But the above-mentioned renal-induced increase in mineralocorticoid secretion requires the integrity of the pituitary. Hypophysectomy greatly reduces renal hypertension(9), probably due to lack of glucocorticoids, since administration of ACTH to hypophysectomized, hypertensive animals restores the hypertension to pre-hypophysectomy levels(10). This indicates that, in cold-exposed, hypophysectomized animals, the lack of glucocorticoids would prevent the secretion of renal pressor substances, which in turn would prevent the increase in mineralocorticoids. Thus the pituitary, which normally has no control over the secretion of mineralocorticoids, may, under certain conditions, exert an indirect influence on their secretion by the adrenal glomerulosa via the adrenal fasciculata and the kidney.

Another explanation of the greater cold-induced hyperexcitability of hypophysectomized rats involves the so-called "normalizing effect" of ACTH itself on brain excitability (5). ACTH would tend to oppose the increase in excitability in normal cold-exposed rats, but would be absent in hypophysectomized ones, thereby allowing a more pronounced central nervous excitatory effect.

Finally, the increased hyperexcitability of hypophysectomized, cold-exposed rats might be attributed to failure of temperature regulation and therefore to greater susceptibility to cold. The effects of hypothermia on seizure threshold in other experimental animals are, however, opposite to those observed in hypophysectomized rats. In hypothermic cats it is impossible to elicit seizures by electrical stimulation of the cerebral cortex with the same

intensity of stimulus adequate for this purpose in cats with normal body temperature (11). Also, while electrical stimulation of the motor cortex elicits peripheral movement of the golden hamster during arousal from hibernation, the threshold declines as the animal warms(12).

Summary. The increased brain excitability observed in cold-exposed, intact rats is not mediated through pituitary hyperfunction, because hypophysectomy increases this effect rather than hindering it. In hypophysectomized, cold-exposed rats, the lowering effects on electroshock seizure threshold of absence of ACTH and mineralocorticoids predominate over the opposite effects of hypothermia and absence of thyroid and glucocorticoid hormones.

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Non-Competitive Non-Inhibitory Interaction Between Hippurate and Creatinine in the Kidney.* (28992)

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Under appropriate conditions, renal excretion of creatinine chromogen in several animal species exceeds its glomerular filtration(1). This phenomenon recently has been confirmed in the dog and has been attributed to renal tubular activity(2,3). Inhibition of creatinine excretion by 4-aminohippurate also has been demonstrated and has been described as a competitive phenomenon(3) on the assumption that creatinine and 4-aminohippurate share a common transport mechanism. The following experiments with renal cortical slices do not confirm the contention that hippurate and creatinine share a single transport mechanism.

Methods. Male albino rabbits weighing from 1-2 kg were decapitated and exsanguinated, and their kidneys were removed. Slices of rabbit kidney cortex were prepared with a Stadie-Riggs microtome and were incubated in a phosphate-buffered saline medium (pH 7.4) at 27°C for one hour in an atmosphere of oxygen(4). At the end of incubation the concentration of creatinine chromogen(5) or of 4-aminohippurate(6) both in slices and in media was determined for calculation of slice to medium concentration ratios.

Results. Concentration ratios for creatinine chromogen. Data are summarized in Table I. Each figure is the arithmetic mean of a total of 9 observations in 3 rabbits. Since acetate and probenecid had no significant influence on the concentration ratios for creatinine chromogen, there is no evidence that creatinine is actively accumulated by renal cortical slices. It is, therefore, unlikely that creatinine and 4-aminohippurate share a single transport mechanism.

Interaction of creatinine chromogen and 4-aminohippurate. Data are summarized in Table I. Since creatinine did not influence

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