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BRIEF COMMUNICATION

Time Course of Protection from Audiogenic Seizures by Glucose and Insulin in Audiogenically Primed C57BL/6J Mice

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SCHREIBER, R. A. *Time course of protection from audiogenic seizures by glucose and insulin in audiogenically primed C57BL/6J mice.* PHARMAC. BIOCHEM. BEHAV. 8(5) 619–621, 1978. – Glucose and insulin injections both decrease seizure susceptibility in C57BL/6J mice subjected to audiogenic priming at 16 days of age and tested at 21 days of age. These data support an hypothesis of a defect in immediately available energy reserves in brains of audiogenic seizure prone mice.

Audiogenic seizures Glucose Insulin Brain energy reserves

IT IS known that prior to the onset of a seizure there is an unusual amount of neuronal activity which requires a considerable amount of energy from chemical sources [1, 2, 3]. There may be a lower limit to the energy level required in brain to subserve organized neural activity. Should this be, then audiogenic-seizure-prone mice may have insufficient reserves in some area(s) of brain to last through an initial few seconds of large external stimulus-induced energy expenditure until energy repletion processes can begin to replace used stores. Non-audiogenic-seizure-prone mice presumably have sufficient reserves, and do not fall below some lower limit necessary to maintain coherent nervous activity.

Preliminary data [5] show that the levels of glycogen and phosphocreatine, the two major energy reserve stores in mammalian brain, are lower during a period of susceptibility to audiogenic seizures (AGS) in DBA/2J mice. Secondly, glucose and insulin injections (both of which would tend to enhance glycogen stores) provide protection from AGS, with a time course consistent with an hypothesis of a transient increase in glycogen stores in brain.

The studies reported here were performed on C57BL/6J mice subjected to audiogenic priming at 16 days of age, and tested at 21 days of age – at peak susceptibility to AGS (SAGS) given the priming conditions used here. These experiments were performed to determine if the previously reported protective effect of glucose and insulin on SAGS was specific for genetically based SAGS, or was general for environmentally-induced SAGS as well.

MATERIALS AND METHODS

Animals

C57BL/6J mice were bred in house, and maintained with ad lib access to Purina Mouse Breeder Blox and tap water. Temperature was maintained at about 23°C; lights were turned on at 0700 and off at 1900.

Audiogenic Seizures: Audiogenic Priming and Testing

Each mouse was placed individually into a 45.72 cm high by 30.45 cm wide chromatography jar enclosed in a sound-attenuating chamber. The mouse was allowed to explore for 15 sec, during which both the lid to which is attached an Edwards No. 340 electric bell generating 127 ± 2 dBA (broad-band) at the level of the mouse was placed on the chromatography jar and also the lid to the sound-attenuating chamber was put in place. For audiogenic priming 16-day-old mice were exposed to the noise for 60 sec [4]. All mice were then returned to their home cages, where they remained until testing at 21 days of age using the same bell. During the testing trail, the latency to and the occurrence of wild running, clonic, tonic and lethal seizures was recorded.

Drugs

D-Glucose was dissolved in H_2O to a concentration of either 10% or 20% (w/v), injections of up to 0.5 ml were administered IP. Insulin (Lilly U–100 R) was diluted in H_2O to 1 U/ml, and either 0.05 or 0.1 ml was injected, SC. Pilot data on larger doses indicated that 1 U/ml was 1/2 the dose at which overt behavioral changes could be detected.

In a first experiment, 21-day-olds were injected IP with 0.2 ml of 10% or 0.5 ml of 10%, or 0.5 ml of 20% glucose, and tested for susceptibility to AGS either 15 min, or 1, 3, 6, or 24 hr later. Glucose would be expected to raise cell glucose levels, and subsequently raise cell glycogen levels.

In a second experiment, either 0.05, 0.10, or 0.2 U of

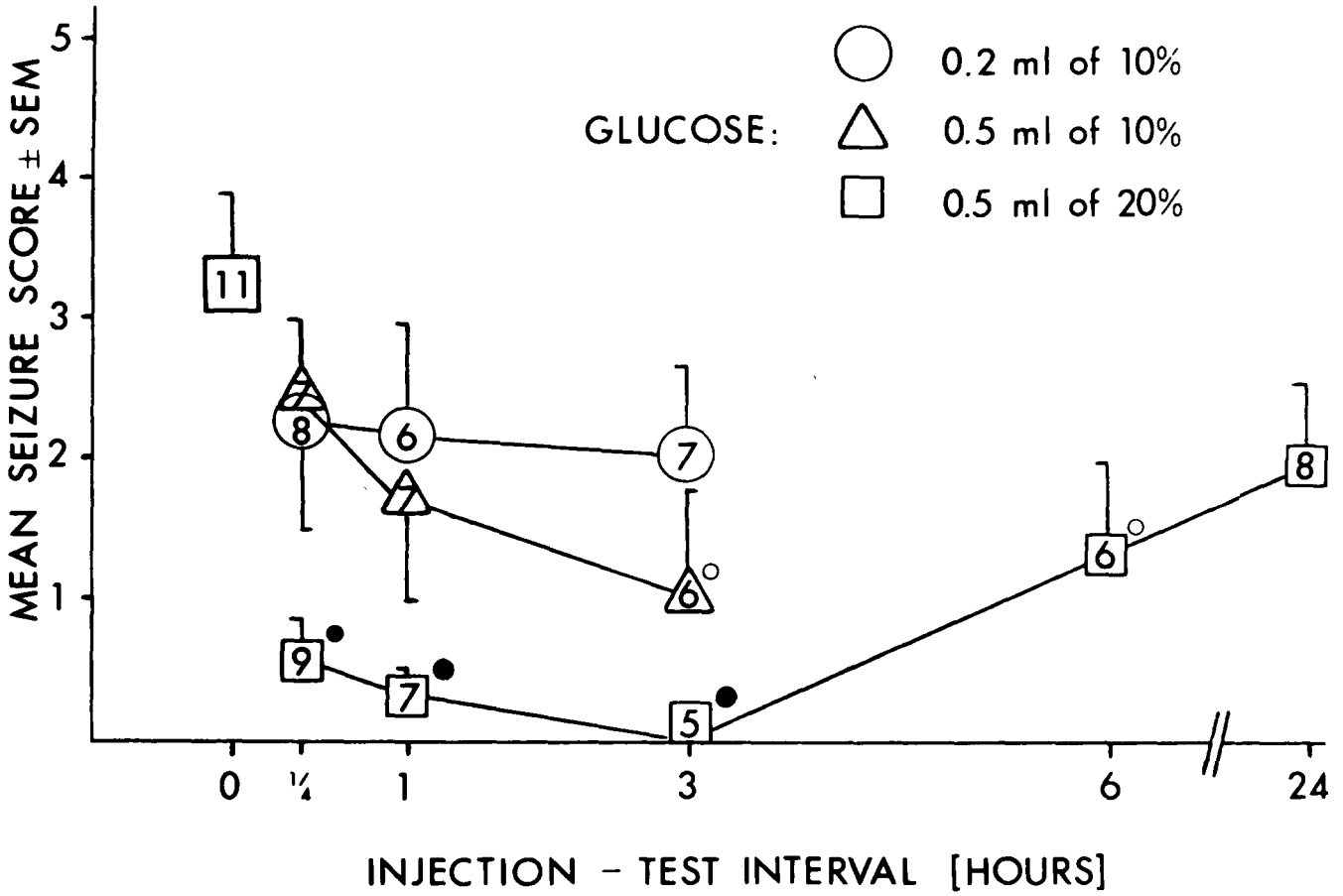

FIG. 1. The time course of protection from audiogenic seizures in 21-day-old C57BL/6J mice. Mice were subjected to 60 sec of 127 dB noise at 16 days of age (audiogenic priming). At 21 days of age, mice were injected IP with varying doses of glucose, and tested for susceptibility to audiogenic seizures at the time indicated. The sham-injected control value is shown at T_0. Mice must wild run prior to entering into a clonic seizure, and have a clonic prior to a tonic seizure. Each mouse was assigned a seizure score according to the most severe seizure level attained: no response, 0; wild run, 1; clonic-flexion, 2; clonic-extension, 3; tonic, 4; and lethal seizure, 5. The mean ± SEM for each group is plotted above. No mouse was tested more than once; the number of mice per group is indicated within the symbol. The statistically significant differences are indicated (student's t: $p<0.05$ by open circles, $p<0.01$ by solid circles).

fasting-acting insulin was injected SC into 21-day-old C57 mice. Insulin would be expected to reduce plasma glucose levels and raise cell glycogen levels.

RESULTS

Experiment 1

There were no differences between injected and controls at any times, with the 0.2 ml of 10% dose. When the level was raised to 0.5 ml of 20% glucose, there were highly significant differences at 15, 60 min and 180 min post-injection (presumably sufficient time for the glucose to be stored as glycogen and therefore to enhance the energy reserve pool in brain), when AGS activity was reduced to zero. Data are shown in Fig. 1.

Experiment 2

Insulin would be expected to reduce plasma glucose levels and raise cell glycogen levels. At 15 and 30 min postinjection, after 0.05 U, there were no significant differences between injecteds and sham-injected controls. At 60 min, almost complete protection was seen, what would be expected if glycogen stores were enhanced, and if glycogen breakdown under stress were also returning to normal as the insulin itself was cleared. By 360 min, AGS levels had returned to control levels. As also seen in Experiment 1, the mice appeared alert and active during the period of anticonvulsant action, much to the contrary of the effects of other anticonvulsant agents. At the 0.2 U dose, however, the mice were almost comatose, and lay in a splayed position at 180 min postinjection. The mice showed no noticeable response to the bell when tested.

DISCUSSION

Preliminary biochemical data show that levels of phosphocreatine and glycogen are lower during the period of susceptibility than afterward in auditory cortex of DBA/2J mice. Secondly, preliminary pharmacological data indicate that glucose or insulin both afford protection from AGS-induction by an acoustic stimulus at the ages of peak susceptibility, without any other overt behavioral consequences usually observed with many anticonvulsant drugs in DBA/2J mice [5].

The two experiments reported here first replicate the

TABLE 1

PROTECTION FROM AUDIOGENIC SEIZURES BY 0.05 U FAST-ACTING INSULIN. SIXTEEN-DAY-OLD C57BL/6J MICE WERE EXPOSED TO 127 dB FOR 1 MIN AND TESTED FOR AUDIOGENIC SEIZURES AT 21 DAYS OF AGE. INSULIN WAS INJECTED SC, AND MICE WERE TESTED AT THE TIMES AFTER INJECTION INDICATED BELOW

	N	WR	C	C^+	T	D	$\bar{X}$	SEM	t vs C	one-tail $p<$
Control	10	10	7	7	7	1	3.20	0.49		
15 min	10	7*	4	4	4	4	2.30	0.74	0.957	0.20
30 min	10	5*	4	4	4	3	2.00	0.76	1.259	0.02
60 min	11	6*	3*	3*	3*	1	1.45	0.57	1.835	0.05
180 min	8	7	4	3	1*	1	2.00	0.56	1.507	0.10
360 min	7	7	4	4	4	1	2.857	0.68	0.386	

WR=wild run, C=clonic-flexion, C^+=clonic-extension, T=tonic, D=lethal seizure.
*Statistically significant differences (2×2 χ^2, corrected for continuity, $p<0.05$).

anticonvulsant effects of glucose and insulin injections, and secondly, show that protection is also afforded to mice made SAGS through audiogenic priming.

One other study has been found by this investigator in which genetically AGS-prone mice were injected with glucose, and later tested for AGS [6], though they performed their research for a different reason. They found a significant reduction in AGS levels at 4 hr postinjection – the only time reported. This is the first report, to this author's knowledge, of glucose and insulin-induced protection for environmentally-induced SAGS. Experiments are under way to determine if drug-withdrawal-induced SAGS is also amenable to protection by these agents.

These data are consistent with an hypothesis of decreased energy reserves in brain during a period characterized by SAGS. If AGS-prone mice do not possess the capability to last through an initial few seconds of large stimulus-induced energy expenditure until energy repletion processes can begin to replace used stores, then perhaps they fall below some lower limit in some area of brain necessary to maintain organized nervous activity. Glucose and insulin both would tend to transiently increase cellular energy reserves, and would therefore be expected to have anticonvulsant activity. These data reported here support this hypothesis.

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