Formalin induced sodium appetite: Dose-response relationships¹

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Abstract

Rats were injected subcutaneously with doses of from .10 to 1.5 ml of 1.5% formalin. Saline intake was measured 24 hr. after injection and was found to increase linearly with formalin dosage. The minimal effective dose was .15 ml, and the maximal effect was reached at a dose of .75 ml.

Problem

It has recently been shown that subcutaneous injections of formalin produce a depletion of body sodium and thereby elicit increased salt intake in the rat (Wolf & Steinbaum, 1965). The mechanism by which formalin produces sodium depletion has been studied in detail (Stricker, 1965), and it has been demonstrated that the resulting appetite is specific for the sodium ion (Handal, 1965).

In a recent study by Wolf & Quartermain (1965) it was found that extremely small doses of formalin are sufficient to potentiate sodium intake when the rats are given a sodium deficient diet and a brief and sensitive testing procedure is used. The present study employs similar experimental conditions to determine (1) minimal and maximal effective doses of formalin and (2) the degree to which predictable changes in sodium appetite can be induced by varying formalin dosage.

Method

Twenty eight male Holtzman Sprague-Dawley rats between 90 and 120 days old approximately 350 gm B.W. were used. They were housed in standard individual cages and given access to 15 m saline at one end of the cage and water at the other end. Purina chow was available ad lib.

After two days on the above regimen (during which time the rats presumably learned the respective positions of the saline and water in the cages) the rats were removed to other individual cages and given water and a sodium deficient diet ad lib. At the end of the first day of sodium deprivation each of the 28 rats was randomly assigned to one of seven groups. Each group received one of the following doses of a 1.5% aqueous formalin solution: 0 ml (sham injection), .10 ml, .15 ml, .20 ml, .25 ml, .75 ml, or 1.5 ml. At the end of the second day rats were returned to the predeprivation cages and given access to .50 m saline and water. The solutions were given in inverted graduated 15 ml centrifuge tubes with standard drinking nozzles and were in the same positions as during the predeprivation period. The .50 m saline concentration was used because this strong solution appears to be unpalatable

to normal rats and thus is not ingested in the absence of a drive.

Readings of saline intake were taken at fixed 15 min. intervals after placing the rats in the cages. Each rat was allowed three additional 15 min. intervals after the first 15 min. interval in which at least .1 ml of saline was ingested. In this way the saline was available to each rat for at least 45 and at most 60 min. after the commencement of drinking. Water intake was measured at the end of the testing period.

Results

Figure 1 shows the saline intake of the individual rats in the seven groups. Increasing doses of formalin produced corresponding increases in saline intake (p for linear trend $< .001$). Comparisons of individual groups by Duncan tests indicated significant ($p < .01$) differences between all adjacent groups except 0 vs. .10, .15 vs. .20, and .75 vs. 1.5.

Water intake also increased with formalin dosage (p for linear trend $< .001$). This increase was undoubtedly secondary to the intake of the highly concentrated saline solution because water was available ad lib prior to the test and presumably, all rats were satiated at the beginning of the test.

Discussion

The results clearly show that the degree of sodium appetite elicited by a given dose of formalin is highly predictable. Under the present conditions the minimal effective dose of 1.5% formalin was .15 ml, and the appetitive effect approached asymptote at .75 ml.

Fig. 1. .50 m saline intake during the 1 hr. testing period as a function of formalin dosage. Each bar represents the score of an individual rat.

Because of the great efficiency of the normal rat's kidney it is difficult to induce sodium deficiency by removal of sodium from the diet. Thus, other methods of sodium depletion have been devised. The most commonly used method of inducing a rapid depletion of body sodium has been intraperitoneal dialysis against glucose (e.g. Smith, Pool, & Weinberg, 1958). Experience in this laboratory indicates that this procedure does not yield the predictable increases in sodium appetite which were demonstrated in the present study by use of the formalin technique, and more importantly, that the dialysis procedure is extremely stressful, often leaving the animal in a debilitated state for hours afterward.

Subcutaneous injection of formalin may also be very stressful. However, the doses used to produce stress effects are much larger than those of the present experiment. For example, Selye (1937) used daily injections of 1 ml of 4.0% formalin in his studies of the general adaptation syndrome. Under the present

conditions the doses of formalin necessary to stimulate sodium appetite were relatively minute. This finding renders the formalin technique much more useful for studies of this drive because the stressful side effects should be essentially negligible.

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Note

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