Sodium intake as a function of the concentration, volume, number, and locus of formalin injections

JOHN J. PORTER, HELMUT RELINGER, and MARIANNE STEELE

University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201

The effects of formalin upon sodium need were investigated by means of six alternate-day subcutaneous injections administered in four distinct sites. Either 1.0 or 2.5 ml of .5% or 1.5% formalin was injected. The effects of these injections upon consumption of .15 M saline over 12 days was examined. In general, only 2.5 ml of 1.5% formalin produced a significant and consistent sodium need over days. Ss in all groups responded to formalin injection by taking in large amounts of sodium 24 h but not 45 h after injection. Distilled water intake in Ss' home cages followed the pattern of saline intake, with hours of measurement contributing to the significant interactions with the other variables. The use of various concentrations of saline as a reinforcer for long-term sodium need was discussed.

Considerable research (cf. Stricker, 1966; Handal, 1965; Wolf & Steinbaum, 1965) has demonstrated that a single injection of a weak formalin solution produces a strong sodium need in the rat. Porter and Relinger (1972) administered several injections at a 48-h interval in order to examine the motivational effects of sodium need sustained over several days. An examination of the research literature yielded no cases where formalin injections were repeated over days, or where the motivational durability of the formalin-produced sodium need was examined. Though Porter and Relinger administered four subcutaneous injections of formalin, the primary effect of the injections upon saline intake seemed to occur following the first injection. Sodium intake was greatly reduced following the next three injections. Several factors may have accounted for the failure to find a consistent effect of repeated formalin injections over 8 days. The present study was addressed to the effects of a weaker saline concentration, clearly differentiated injection sites, and the effects of different volumes and concentrations of formalin upon the durability of the sodium need.

Using a .5-M solution, Handal (1965) found asymptotic saline intake with the injection of .75 ml of formalin. With a .33-M saline solution, Porter and Relinger found significantly greater intake after the injection of 1.0 ml of formalin than after .75 ml. These authors suggested that a small change in the volume of .5-M saline ingested would compensate for differences in sodium need produced by injection of .75 or 1.0 ml of formalin, Handal's largest injections. Thus, small changes in the intake of a strong saline solution after different formalin injection concentration and volume would make it difficult to detect differences in sodium need. If weaker saline solutions were used, a substantially larger intake would be needed to compensate for differences in sodium need. A study by Jalowiec and Stricker (1970) provided evidence for this view in that their Ss required far less .51-M saline to restore their sodium balance than was the case with .15-M or .075-M saline. Following this reasoning, Porter and Relinger's .33-M saline

concentration may have been too concentrated to adequately measure possible differences in sodium need after successive injections. Thus, the present study used isotonic (.15 M) saline throughout.

In this experiment, Ss received either 2.5 or 1.0 ml of formalin with the intent that a larger dosage would more convincingly demonstrate a sodium need after successive injections. Successive injection sites were separated by 2.5 cm in order to minimize the skin irritation observed in our previous study—a possible source of the weak effect of later injections. In addition, the injections were programmed such that Injections 5 and 6 were administered at the initial two sites in order to examine the effect of an 8-day rest interval upon healing of the injection sites and possible variations in the sodium need produced by reinjection at Sites 1 and 2.

Porter and Relinger were successful in eliciting an apparent sodium need with a .5% formalin solution that produced far less skin irritation than the 1.5% dosage. In the course of pilot research which followed, an attempt was made to elicit sodium need by injecting .3% or .1% formalin using .15-M saline as a reward. At these concentrations, saline intake did not exceed that of a control group; as a result, the present study returned to injections of .5% or 1.5% formalin.

METHOD

Subjects and Design

Twenty-five male Holtzman albino rats, approximately 140 days old when training began and weighing from 370 to 425 g, were used. An equal number of Ss were assigned to five groups matched for body weight. The four experimental groups received injections of 1.0 or 2.5 ml of either 1.5% or .5% formalin every 48 h over a 12-day period. A control group received the same pattern of injections, but of 2.0 ml of .15-M saline.

Procedure

On Days 1-5, all Ss were exposed for 1 h daily to test cages containing a water bottle filled with distilled water and a 100-ml graduated drinking tube filled with .15-M saline. Both containers were located on the front wall of the test cages and spaced 7 cm

Fig. 1. Mean daily saline intake for the four experimental and one control group. The first figure in the legend is the amount injected, the second figure is the injection concentration.

apart. This procedure ensured that Ss were familiar with the locations of water and saline in the test cages.

After testing on Day 5, Ss were returned to their home cages, where they had access to distilled water and a modified sodium-deficient diet (Nutritional Biochemicals Co.) ad lib. This regimen was followed throughout the remainder of the experiment. Three hours after testing on Day 5, all Ss received their first injection while lightly anesthetized with ether. Successive injections were administered subcutaneously at four clearly differentiated sites, 2.5 cm apart, along the midline of the S's back. In order to examine the effect of reinjection, injections 5 and 6 were administered at Sites 1 and 2. Testing occurred 24 and 45 h after injection. Saline and water intake were recorded after each test session, as was home cage water intake. Ss were tested 3 h before their next injection in order to minimize the possibility of a conditioned aversive reaction to saline as demonstrated by Woods, Weisinger, and Wald (1971).

RESULTS

Figure 1 presents the daily saline intake for the experimental and control groups over the 12 days of the experiment. The daily saline intake of the control group was close to zero throughout the experiment and thus this data was not treated in the report of saline intake.

The 1.5% groups consistently drank more than the .5% groups [$F(1,16) = 15.98, p < .01$]. Similarly, the injection of 2.5 ml of formalin produced significantly greater saline intake than 1.0 ml [$F(1,16) = 10.67, p < .01$]. Simple effects tests indicated that the significant interaction between percentage formalin (PF) and amount injected (AI) [$F(1,16) = 5.12, p < .05$] was due to the large amount of saline ingested by the 2.5-ml/1.5% group ($p < .01$).

As was the case in Porter and Ringer's (1972) study, Ss drank much greater quantities of saline when tested 24 h after injection than during the 45-h test [$F(1,16) = 216.10, p < .01$]. Significant interactions between hour of measurement (HM) and PF [$F(1,16) = 53.25, p < .01$], HM and AI [$F(1,16) = 28.52, p < .01$], and the triple interaction of these variables [$F(1,16) = 18.82, p < .01$] all clearly resulted from the much larger

volume of saline taken in 24 h after injection compared to the relatively minute saline intake when tested 45 h after injection. These observations were confirmed by simple effects tests which demonstrated uniform significant effects of PF and AI 24 h after injection ($p < .01$), but no significant differences due to these factors at the 45-h test. The small saline intake at the 45-h test appeared to be due to the reduced sodium need from the 1-h intake period 21 h earlier and the reabsorption of the edema.

There was a significant decline in the amount of saline ingested over days (D) [$F(5,80) = 12.47, p < .01$], though the pattern of decline was not uniform over groups. As can be seen in Fig. 1, the groups receiving the 1.5% formalin tended to show higher performance than the .5% groups throughout training [$F(5,80) = 3.94, p < .01$], while the superior saline intake of the 2.5-ml/.5% group compared to the 1.0-ml/1.5% group, starting on Day 7 and ending on Day 11, appeared to be the source of the triple interaction between D, AI, and PF [$F(5,80) = 3.92, p < .01$].

The significant interaction between HM and D [$F(5,80) = 7.25, p < .01$] was due to the significant decrease over days in the amount of saline ingested 24 h after injection ($p < .01$), whereas there was little change in the minor saline intake over days when measured 45 h after injection. Finally, the triple interaction between HM, PF, and D [$F(5,80) = 3.19, p < .05$] again appeared to be due to the greater intake of the group injected with 2.5 ml of 1.5% formalin.

Water Intake

While there were no significant differences in distilled water intake between groups in their test cages, the pattern of home cage water intake, illustrated in Fig. 2, tended to follow the daily saline intake pattern, though the effects were clearly of a lesser magnitude. The data of the two .5% formalin groups overlapped that of the

Fig. 2. Mean daily home cage water intake for the two groups receiving 1.5% formalin and the saline control group. The two .5% groups overlapped the water intake of the 1.0-ml/1.5% group and the control group. For graphical clarity, the latter two groups are omitted.

control group and that of Group 1.0 ml/1.5%. For graphical clarity, only the groups receiving 1.0 and 2.5 ml of 1.5% formalin and the control group were included in Fig. 2. The main effects of PF and AI were not significant, and the interaction of these variables did not quite reach the .05 level of confidence [$F = 4.30$, F of 4.49 required]. However, HM did interact significantly with PF [$F(1,16) = 26.66$, $p < .01$] and with AI [$F(1,16) = 19.62$, $p < .01$]; and the triple interaction of these variables was significant [$F(1,16) = 7.05$, $p < .05$]. Water intake measured 24 h after injection was clearly greater than 45 h after injection, leading to a significant HM effect [$F(1,16) = 153.48$, $p < .01$]. Simple effects tests of the interaction between HM and other variables demonstrated that 24 h after injection the 2.5-ml/1.5% group had significantly greater water intake ($p < .01$) than any other group and that the other experimental groups did not differ significantly among themselves. The control group water intake was significantly below that of Group 2.5 ml/1.5%, but did not differ reliably from the other groups.

Overall, there was a significant decline in home cage water intake over days [$F(5,80) = 11.62$, $p < .01$] and a significant interaction between HM, AI, and D [$F(5,80) = 3.25$, $p < .05$].

DISCUSSION

The 2.5-ml/1.5% group was superior in saline intake to the other groups throughout the 12 days of this study. This group showed only a mild decline in saline intake over successive injections compared to the other groups and, with the exception of performance after Injection 5, this group maintained a reasonably large and consistent saline intake. The 2.5-ml/1.5% group seemed an optimum combination since much stronger concentrations would risk unduly severe stress (cf. Selye,

1937) and the injection of greater volume of formalin would produce an edema covering too extensive an area for repeated injections at discrete sites.

Any conclusion about the correct volume/concentration of formalin injections remains tentative until the effects of variation in the temporal interval between formalin injections has been investigated. Thus injections administered every 24 h might indicate that more frequent injections lead to a more constant sodium need than do 48-h injections. On the other hand, injections at more widely spaced intervals, say every 72 h, might reduce the irritation generally observed with 2.5 ml of 1.5% formalin. At either injection interval, the effects of permitting Ss to ingest various controlled amounts of saline upon motivation in an instrumental situation (cf. Quartermain, Miller, & Wolf, 1967) over several injections should be investigated before using formalin as a source of motivation in instrumental behavior.

It is important to note that the use of .15-M saline as reinforcement in order to get a more detailed picture of differences in sodium need seemed effective in extending maximum saline intake. Thus, with equivalent injection concentrations (1.0 ml of 1.5% formalin) and saline intake measured 24 h after injection, Handal's (1965) Ss drank approximately 9 ml of .5-M saline, Porter and Relinger's Ss drank approximately 24 ml of .33-M saline, and in the present study Ss drank approximately 33 ml of .15-M saline.

Turning to water intake, the pattern of water intake observed in Ss' home cages clearly followed apparent sodium need and confirmed the observation (cf. Stricker, 1966) that a decrease in extracellular fluid volume elicits water intake. The similar patterning of water and saline intake following formalin injections, even though water intake does not correct the deficit in extracellular fluid volume, presents intriguing

possibilities for instrumental and operant rewards without need reduction. These possibilities will be explored in a later series of experiments.

REFERENCES

- Handal, P. J. Formalin-induced sodium appetite: Dose-response relationships. *Psychonomic Science*, 1965, 3, 511-512.
- Jalowiec, J. E., & Stricker, E. M. Restoration of body fluid balance following acute sodium deficiency in rats. *Journal of Comparative & Physiological Psychology*, 1970, 70, 94-102.
- Porter, J. J., & Relinger, H. Daily sodium intake as a function of time of measurement and formalin injection volume and concentration. *Psychonomic Science*, 1972, 26, 276-278.
- Quartermain, D., Miller, N. E., & Wolf, G. Role of experience in relationship between sodium deficiency and rate of bar pressing. *Journal of Comparative & Physiological Psychology*, 1967, 63, 417-420.
- Selye, H. Studies on adaptation. *Endocrinology*, 1937, 21, 169-188.
- Stricker, E. M. Extracellular fluid volume and thirst. *American Journal of Physiology*, 1966, 211, 232-238.
- Wolf, G., & Steinbaum, E. A. Sodium appetite elicited by subcutaneous formalin: Mechanisms of action. *Journal of Comparative & Physiological Psychology*, 1965, 59, 335-339.
- Woods, S. C., Weisinger, R. S., & Wald, B. A. Conditioned aversion produced by subcutaneous injection of formalin in rats. *Journal of Comparative & Physiological Psychology*, 1971, 77, 410-415.

(Received for publication September 29, 1972;
accepted October 17, 1972.)