Electroconvulsive shock impedes the learning of taste aversions: Absence of blood-brain-barrier involvement*

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Rats made ill by lithium chloride injection 30 min after drinking saccharin-flavored water avoided that flavor upon subsequent encounter unless ECS was interpolated during the taste-illness interval. Preinjection of animals with trypan blue, a supravital dye known to mitigate dysrhythmic effects of ECS on brain EEG and thought to do so by preventing increased permeability of the blood brain barrier (BBB), did not prevent the disruptive effect of ECS on avoidance learning. Interpretation of the data suggested that increased permeability of the BBB may not be the mechanism of an ECS-induced decrement in taste avoidance learning.

Taste aversions were conditioned in a single trial, despite taste (CS)-illness (US) intervals of several hours (Garcia & Ervin, 1968). Interpolation of electroconvulsive shock (ECS) during the CS-US interval impeded avoidance learning (Kral, 1970), but the mechanism of ECS action remained unknown. Numerous *in vivo* studies indicated that the permeability of the blood brain barrier (BBB) was markedly increased following ECS (Aird, 1958; Aird, Strait, Pace, Hrenoff, & Bowditch, 1956a) and that this permeability as well as the dysrhythmic effects of ECS on brain EEG were prevented by pretreatment of experimental animals with a supravital dye (Aird & Strait, 1944; Aird, Strait, Pace, Hrenoff, & Bowditch, 1956b). The protective effect of the dye led Aird (1958) to suggest that the dysrhythmic effect of ECS was due to the increased permeability of the BBB. The purpose of this study was to investigate the possibility that increased BBB permeability was a determinant of ECS-induced impedance of taste aversion learning. It was hypothesized that pretreatment of rats with trypan blue would mitigate the reported effects of interpolated ECS.

MATERIAL AND METHODS

Forty male Sprague-Dawley rats weighing 300-400 g were divided randomly into four equal groups and individually housed. Throughout the experiment, the animals were fed chow *ad lib* but water drinking was restricted to 10 min/day. From the second to the fourth day of the drinking schedule, rats in three groups received intraperitoneally (IP) 3 ml of a

2% (w/v) trypan blue solution/kg body weight daily. The fourth group received equivalent volumes of 0.9% saline (placebo) on the same injection schedule as those receiving the dye. On the fifth day (acquisition trial), all animals were allowed to drink a sweet aqueous solution of 0.1% (w/v) saccharin (CS) for 10 min. Thirty minutes later (CS-US interval), they received IP 10 ml/kg of a 0.4M solution of lithium chloride (US) or an equivalent volume of 0.9% saline. Lithium chloride is known to induce a strong taste aversion when paired with a distinctive flavor (Kral, *in press*). At

the midpoint of the interstimulus interval (15 min after CS), each animal, depending on group, received ECS (60 mA for 0.6 sec) or sham shock (no current passed). ECS apparatus and procedures are described elsewhere (Kral, *in press*).

Individual group conditions were as follows: The conditioning control (CS-US) group was pretreated with dye, drank sweet water, was sham shocked, and was injected with lithium chloride (LiCl). This group was considered an appropriate comparison with which to assess ECS-induced learning deficits in the experimental groups. A nonconditioning control (CS-saline) group received the same treatment as CS-US, except that the rats received ECS and subsequently were injected with 0.9% saline. This group provided a baseline of normal sweet-water consumption. Experimental groups, dye-ECS and saline-ECS, were pretreated with dye and 0.9% saline, respectively. Both groups received sweet water, ECS, and lithium injections.

A test trial involving only the presentation of the CS for 10 min was carried out 3 days following the acquisition trial. The long acquisition-test interval allowed time for recovery from LiCl-induced toxicosis, which was observed as a return to baseline levels of water consumption. While on schedule, animals drank from graduated cylinders. Animals in all groups except

Fig. 1. Means and SDs of sweet water intake on acquisition and test day for all groups. A strong conditioned taste aversion (CS-US) was mitigated by interpolated ECS (saline-ECS), but this effect was not influenced by pre-ECS injection of trypan blue (dye-ECS).

*This work was supported by Grant PHS HD-00183 and State of Kansas Grant No. 0421-6-629-0101.

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CS-saline received an injection of trypan blue 24 h after test day (2 h prior to decapitation). The brains were removed immediately thereafter and macroscopically inspected for signs of dye coloration.

A simple randomized analysis of variance was performed on acquisition and test trial intake scores, and Duncan's multiple range test was used to make two-sample comparisons.

RESULTS

Inspection of Fig. 1 indicates that sweet water intake was not significantly different across groups on acquisition day ($F < 1$) but became highly so on test day ($F = 96.40$, $df = 3/33$, $p < .001$).

Intake was greatly depressed among CS-US animals compared to CS-saline ($p < .01$), indicating a strong conditioned taste aversion. The dye-ECS and saline-ECS groups occupied intermediate positions on intake, differing significantly both from CS-US (conditioned) and CS-saline (nonconditioned) animals ($p < .01$), indicating a reduction in the strength of the aversion. They did not, however, differ among themselves ($p > .05$). The validity of this observation is strengthened by the absence of floor and ceiling effects.

The brains (both whole and gross cross sectioned) of nondye-pretreated rats (saline-ECS) which received ECS were stained blue following posttest-trial dye injection. Such coloration, however, did not appear in the brains of dye pretreated rats whether they were convulsed (dye-ECS) or not (CS-US), indicating no significant increase in BBB permeability among these animals.

DISCUSSION

Pretreatment of rats with a supravital dye (trypan blue) should prevent or mitigate the known disruptive effect of interpolated ECS on taste aversion learning (Kral, 1970)

if increased BBB permeability is a significant determinant in the mechanism of ECS action. This hypothesis was based on a series of studies stemming from an interest in the possible role of the BBB in such clinical conditions as epilepsy, cerebral concussion, and electrically induced convulsions. After concussion (Aird, Strait, Zealear, & Hrenoff, 1952), cold-induced localized cerebral lesion (Flodmark, 1965), and ECS (Aird et al, 1956a; Aird, 1958), the systemic penetration of the brain by drugs with only limited access to it under normal barrier conditions was found to be increased, suggesting that the BBB mechanism was disturbed. In all cases, altered CNS physiology was indicated by the development of EEG dysrhythmias. Repeating the same experiments after injection of a supravital dye (Aird et al, 1956; Aird, 1958) indicated that the dye prevented both the BBB and physiological changes. The brain waves remained essentially normal after concussion or electric shock, provided the dye was injected beforehand.

The present results did not support a BBB permeability hypothesis of shock action in that ECS-induced impedance of avoidance learning was unattenuated despite pre-ECS injection of experimental animals with trypan blue. The brains of dye-pretreated rats (dye-ECS) did not stain in contrast to nondye-pretreated rats (saline-ECS) following posttest injection of trypan blue, thus indicating that permeability of the BBB of dye-ECS animals might not have been appreciably altered by ECS. Trypan blue is unable to pass from blood to brain under normal barrier conditions but can pass with any impairment of the BBB (Steinwall, 1961; Flodmark, 1965). The present data suggest that permeability changes in the BBB are probably not

significant determinants in the mechanism of ECS-induced impairment in the learning of taste aversions. However, more quantitative measurements of BBB permeability will be required before this hypothesis of ECS action can be ruled out.

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