Altered Effect of Potassium Ions on Cerebral Respiration in vitro Following Subcortical Lesions

Abstract. *The rate of glucose-supported respiration in cortical slices taken from control rats and rats with bilateral lesions in the caudate nucleus was increased by 92 to 95 percent after the addition of potassium chloride. In cortical slices taken from rats with bilateral lesions in the septal area the respiratory rate increased by only 40 percent.*

Rats with bilateral lesions in the septal area have been reported to demonstrate an increased sensitivity to barbiturates (*1*, *2*). As part of an investigation of this increased sensitivity, we have examined the possible effects of septal lesions on cerebral metabolism. Jowett and Quastel (*3*) suggested that the action of barbiturates is related to their depression of oxidative processes in the brain. The respiration of cerebral slices can be increased in vitro by the addition of potassium chloride; such potassium-stimulated respiration is highly sensitive to depression by barbiturates (*4*). We have therefore examined the effects of lesions in the septal area on the duration of sleep induced by barbiturates and the effects of these lesions on the respiration of slices of cerebral cortex in vitro both before and after the addition of potassium chloride.

Male albino rats of the Sprague-Dawley strain (Holtzman) were used. When the rats were 83 to 84 days old, bilateral lesions were produced in the septal area or the caudate nucleus by means of a Krieg-Johnson stereotaxic apparatus in a manner previously described (*2*). Rats with a sham operation and rats not operated on served as controls. In the sham operation, animals were treated in the same manner as those receiving lesions, except that the electrode was never introduced into the brain. Two weeks after surgery, thiopental sodium (20 mg per kilogram of body weight) dissolved in distilled water was injected intravenously and the duration of sleep was determined for all animals by the loss and recovery of the righting reflex (*1*, *2*). The respiratory studies were completed within 2 to 6 weeks after the injection of barbiturate. The animals were killed by decapitation; within 2 minutes after death the brains were removed and placed in ice cold buffer. Four slices were cut freehand from the cerebral cortex of each animal and the remaining part of the brain was placed in 10 percent formaldehyde solution for subsequent histological examination of the locus and extent of the lesion. The cortical slices were incubated in an atmosphere of oxygen at 37°C in a modified Krebs-Ringer phosphate buffer (*5*) containing glucose (2 mg/ml) as substrate. The buffer was modified only by reducing the concentration of calcium chloride to 2.01 m*M*. Oxygen uptake was measured by the direct method of Warburg (*5*). The time that elapsed between death of the animal and the first manometric reading was approximately 30 minutes. Readings were then taken every 10 minutes. After 30 minutes (during which time the respiratory rate was constant) potassium chloride was tipped in from a side arm to bring the final concentration to 106 m*M*. After an equilibration period of 10 minutes, the manometric readings were continued for 80 minutes. The brain slices were then removed, rinsed in distilled water and dried at 80°C for 24 hours. The dried tissue ranged in weight from 1.9 to 5.9 mg. Respiratory rates were expressed as microliters of oxygen consumed per milligram of dry weight per hour.

After the injection of thiopental sodium (Table 1), rats with lesions in the septal area slept significantly longer than the control rats or rats with lesions in the caudate nucleus. These results confirm previous findings from this laboratory, both with respect to the anatomical locus of destruction as revealed by histological examination of the brains and with respect to the effects of the lesions on the duration of sleep induced by a barbiturate (*1*, *2*).

The lesions in the septal area affected both pre- and postcommissural elements of this region. The precommissural septum, a basal telencephalic area lying between the frontal horns of the lateral ventricles, rostral and dorsal to the anterior commissure, and caudal to the genu of the corpus callosum was consistently ablated. Thus the medial and lateral septal nuclei, the dorsal part of the nucleus of the diagonal band, the nucleus septohippocampalis, the dorsal portion of the nucleus accumbens, the precommissural fornix, and Zuckerkandl's olfactory bundle were totally destroyed by the lesion. The postcommissural fornix, ventral hippocampal commissure, posterior septal nucleus, and the bed nucleus of the stria terminalis were always damaged subtotally by the posterior extension of the lesion. Occasionally, the lesions invaded portions of the caudate nucleus immediately adjacent to the lateral ventricle, but this damage was slight and always unilateral. The lesions in the caudate nucleus were placed primarily at the level of the precommissural fornix and ablated the medial portions of the caudate nucleus lying on the borders of the lateral ventricles.

Prior to the addition of potassium chloride there were no detectable ef-

Table 1. Effect of lesions in the central nervous system on the duration of sleeping induced by thiopental and on the respiration of cerebral cortical slices in vitro before and after the addition of potassium chloride. Respiratory rates represent means of three readings over a 30-minute interval before and after the addition of potassium chloride. Since there were no consistent or significant differences in the response of the four cortical slices taken from the same animal, each animal is represented by an average rate obtained from four flasks.

Group	Sleep time			Mean respiratory rates of cerebral slices		Increase in respiratory rates (%)	
	n	Mean (min)	Ratio*	Buffer only (6m*M* KCl)	After addition of potassium (106 m*M*)	Mean	Range
Unoperated	6	13.14	1.00	9.9	19.3	95	62–127
Sham-operated	4	13.35	1.02	10.8	20.7	93	61–113
Caudate lesions	5	15.85	1.21	9.5	17.5	92	53–162
Septal lesions	4	73.81†	5.62	10.6	14.6†	40†	37–43

* Ratio of mean sleep time of operated group to mean sleep time of unoperated group. † A mean value significantly different from the mean of unoperated controls ($p < .05$). In each case there were no overlapping values.

fects of lesions in the central nervous system on the respiratory rates of cortical slices (Table 1). As previously reported (*4*), addition of potassium produced an increase of respiratory rate in all animals. Cortical slices taken from rats with sham operations or rats with lesions in the caudate nucleus responded in the same manner as slices from normal control rats (92 to 95 percent stimulation). However, cortical slices taken from rats with lesions in the septal area showed only a 40 percent stimulation following the addition of potassium chloride. These results were reproduced in three separate experiments in which fresh groups of rats were used.

It is clear that a subcortical lesion can produce significant changes in the metabolic response of cortical tissue in vitro. Although previous work in this laboratory has revealed a variety of behavioral, pharmacological, and biochemical changes following lesions in the septal area of the rat (*1, 2, 6*), the experimental literature provides no clear evidence for either anatomical or electrophysiological changes in cerebral cortex following such lesions (*7*). Therefore, it is not clear whether the effects on cerebral metabolism reported here represent some functional change in the intact animal which might account for the increased sensitivity to barbiturates.

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Intracranial Reinforcement Compared with Sugar-Water Reinforcement

Abstract. *Three ways in which electrical, intracranial reinforcement is reputed to differ from conventional reinforcement were tested in an experiment which equated the form of the responses being reinforced and the response-reinforcement relation. Four groups of rats performed instrumental or consummatory responses reinforced by intracranial reinforcement or sugar. In no comparison did the kind of reinforcement produce a difference, but in every comparison the kind of response reinforced did produce a difference. It is inferred that reputed differences between intracranial reinforcement and conventional reinforcement are artifacts.*

Responses maintained by intracranial reinforcement (ICR) are remarkably rapid and persistent, cease abruptly when reinforcement is terminated, and are peculiarly dependent upon reinforcement of each response. These three generalizations have been made repeatedly and now appear in textbooks (*1*). The statements are based on comparisons between responses for ICR and those for conventional incentives (for example, food) in what is schematically the same apparatus; however, they overlook one, possibly crucial, difference in the temporal-spatial relation between the response and reinforcement. The most common experimental procedure followed with ICR places the subject in an apparatus that delivers ICR as the subject depresses a lever. For conventional reinforcement, the lever press is but one of a chain of responses preceding the reward. Consideration of the distinction leads to the realization that the lever press for ICR is temporally similar to a consummatory response whereas the lever press for food is instrumental. The reports comparing ICR with conventional reinforcement and implying them to have different effects have confounded the variables of kind of reward with delay of reward.

In our experiment we arranged the contingencies of response and reinforcement in two ways. One arrangement required the subject to press a lever as the means of making ICR or sugar water available a little distance away at a dipper cup—the arrangement typical of conventional reinforcement. The other arrangement made ICR or sugar water available immediately when the subject touched the cup in accepting the different incentives—an arrangement analogous to the typical ICR study.

Four albino rats, weighing 250 g each, were randomly assigned to each of four experimental groups. All were brought to 85 percent of their normal body weight and maintained at that weight by limited feeding after each daily experimental session. The subjects scheduled for ICR were fitted with permanent indwelling bipolar electrodes (*2*) with the stimulating tip near the median forebrain bundle and lateral to the ventromedial nucleus of the hypothalamus (*3*).

The experimental chamber was 25 by 25 by 42 cm. A Lehigh Valley retractable lever and a liquid dipper aperture were on one wall. Outside the wall, two liquid dippers were placed so that either (one with a 25 percent solution of sugar water and one dry) could be slid into position under the aperture. A circuit for sensing contacts between the rat and a metal conductor was connected to each dipper. An isolated source of 60-cycle alternating current of 50 to 70 μa, individually adjusted for each rat, served as the ICR.

For two of the experimental conditions, a lever press made reinforcement available at the dipper. For one of these, the sugar-contingent (S-C arrangement), the lever press activated the dipper, filling it with sugar water. Additional pressing accomplished nothing until the subject had contacted the dipper. In the other arrangement, the ICR-contingent (ICR-C), the lever press activated the dry dipper and armed the ICR circuit, thus permitting the subject to obtain reinforcement by contacting the dipper. Since about 1 second was needed for the subject to drink a dipper of sugar water, ICR was made available for a total of 1 second. Multiple trains of stimulation were possible during this 1 second because each contact produced ICR.

In the second pair of conditions, the subject had merely to lap sugar