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Effect of Acorus Oil *in Vitro* on the Respiration of Rat Brain

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By Warburg manometric technique, the steam volatile oil of *Acorus calamus* L. was found to inhibit the oxygen uptake of rat brain homogenate in phosphate buffer. It produced a mild, moderate, and marked inhibition with sodium succinate, sodium glutamate, and glucose, respectively, as respiratory substrates. Lysergic acid diethylamide exhibited very little inhibition on the endogenous respiration and also partially reduced the inhibition caused by acorus oil. In combination with 5-hydroxytryptamine, the inhibitory action of the drug was considerably potentiated.

ACORUS CALAMUS L., is a popular drug in the Ayurvedic system of medicine in India and is widely used for the treatment of various nervous disorders (1). Agarwal, *et al.* (2), in 1956 reported that the total extract of the rhizomes of the plant possessed sedative and analgesic properties. The recent detailed pharmacological investigations carried out by Dandiya, *et al.* (3, 4), showed that the activity of the drug was due to the steam volatile oil. Their main findings are that the oil potentiated the hypnotic activity of barbiturates and ethanol, exacerbated the tonic seizures provoked by convulsive doses of metrazol, reduced the amphetamine toxicity, and caused hypothermia.

It is known that certain drugs produce effects on the oxidative process of the brain *in vitro* and

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that the *in vivo* mechanism can partly be explained in terms of the effects on the enzymatic process (5). Since Dandiya, *et al.*, have suggested that the acorus oil acts similarly to reserpine and also that the mechanism of action is common to that of some ataractic agents, it was considered of great interest to evaluate the mode of action of acorus oil *in vitro* using various substrates and other drugs in combination.

MATERIALS AND METHODS

Albino rats weighing 100–150 Gm. were decapitated and the brain was immediately removed, weighed, washed, and transferred to a previously chilled glass tissue grinder containing Krebs-Ringer phosphate buffer, pH 7.3 (6). The brain was homogenized and the homogenate was diluted with phosphate buffer so that each ml. of the homogenate contained 100 mg. of the fresh tissue.

Oxygen uptake was measured at ten-minute intervals by the Warburg manometric method. The gas phase was air and the temperature of the bath was 38°. Each reaction vessel contained 1 ml. of the homogenate and the solvent control (0.3 ml.) with or without the drug. In those experiments where substrates were used, 0.3 ml. of glucose (0.33 *M*), or 0.3 ml. of sodium glutamate (2.0 *M*), or 0.3 ml. of sodium succinate (0.33 *M*), all dissolved in phosphate buffer, was added. In some experiments 0.3 ml. of 10^{-3} *M* lysergic acid diethylamide (LSD-25) or 0.3 ml. of 10^{-2} *M* 5-hydroxytryptamine (5-HT), in phosphate buffer, was added. The total volume of the fluid in the flask was always made up to 3 ml. with phosphate buffer. The central well of the flask contained 0.2 ml. of 20% potassium hydroxide absorbed on filter paper. The drug with or without substrates was tipped in from the side arm at zero time after ten minutes of equilibration. All the measurements were performed in triplicate and the rate of oxygen uptake, Q_{O_2}, for the first sixty minutes was calculated as μL. of oxygen per hour per 100 mg. of the brain tissue.

The acorus oil was suspended in phosphate buffer containing 1% Tween 80 which also served as control throughout the investigation. The effect of solvent control was found to be insignificant and hence omitted from the text. All the concentrations of drug refer to the final concentration in the reaction vessel.

RESULTS AND DISCUSSION

The effect of various concentrations of acorus oil on the respiration of rat brain homogenate in phosphate buffer is given in Table I. Although the brain homogenate used for each concentration in different experiments varied, the comparison of effect was made on the same batch. Hence, the percentage effect remains within the experimental limits. Acorus oil caused varying degrees of respiratory inhibition with change in concentration: 20% effect was noted with 1:16,000, whereas in concentration of 1:500, it exhibited 70% inhibition.

The ED_{50} (with 19/20 confidence limits) calculated by the method of Litchfield and Wilcoxon (7) was found to be 1:1,980 (1:1,309–1:2,594). This optimum concentration (1:2,000) was selected for studying the effect on respiration in the presence of various substrates and in combination with LSD-25 and 5-HT to find out the influence exerted by them on its activity.

TABLE I.—EFFECT OF VARIOUS CONCENTRATIONS OF ACORUS OIL ON THE OXYGEN UPTAKE OF RAT BRAIN HOMOGENATE IN PHOSPHATE BUFFER

Concentration of Drug	Q_{O_2}[a] without Drug	Q_{O_2}[a] with Drug	Inhibition, %
1:16,000 (3)[b]	69.7 ± 2.5	56.0 ± 2.0	19.7 ± 0.7
1:8,000 (5)	69.7 ± 1.5	47.3 ± 1.7	29.3 ± 1.0
1:4,000 (4)	69.7 ± 2.9	41.8 ± 1.0	41.0 ± 1.2
1:2,000 (6)	70.9 ± 3.1	36.8 ± 1.8	47.7 ± 2.7
1:1,000 (4)	62.2 ± 2.8	25.5 ± 0.7	57.0 ± 2.4
1:500 (3)	52.3 ± 1.0	15.3 ± 0.3	71.3 ± 0.8

[a] Calculated as μL./hr./100 mg. of brain tissue.
[b] Figures within parentheses show the number of experiments in each study.

The rates of oxygen uptake at different intervals by 100 mg. of the rat brain homogenate in the presence of glucose (0.033 *M*), sodium glutamate (0.2 *M*), and sodium succinate (0.033 *M*), with and without acorus oil, are plotted in Fig. 1. Each reading is the average of 12 to 21 reaction vessels in several experiments and is within the variable range of ±5.

It was found that the percentage inhibition in glucose, sodium glutamate, and sodium succinate was 43.1 ± 2.9, 34.1 ± 1.5, and 6.7 ± 1.5, respectively. The inhibition was maximum in glucose and almost negligible in sodium succinate. It is suggested that the action of acorus oil might be localized in the citric acid cycle.

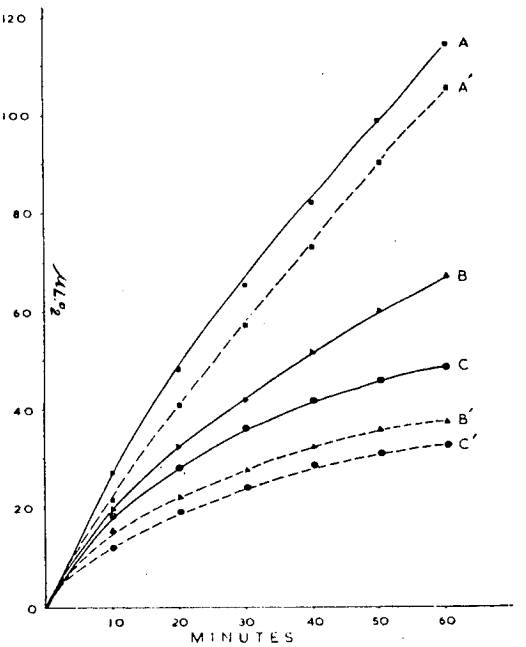

Fig. 1.—The effect of acorus oil (1:2,000) on oxygen uptake of rat brain homogenate in various substrates. *A*, Sodium succinate (0.033 *M*); *A'* sodium succinate + acorus oil; *B*, glucose (0.033 *M*); *B'*, glucose + acorus oil; *C*, sodium glutamate (0.2 *M*); *C'*, sodium glutamate + acorus oil.

TABLE II.—EFFECT OF LYSERGIC ACID DIETHYLAMIDE AND 5-HYDROXYTRYPTAMINE WITH OR WITHOUT ACORUS OIL ON THE RESPIRATION OF RAT BRAIN

Drug	Q_{O_2} in μL./hr./100 mg. of Brain Tissue	Inhibition, %	
Control	60.3 ± 2.6 (7)[a]	...	
Acorus oil (2×10^{-3})	32.3 ± 2.0 $P < 0.001$[b] (7)	46.5 ± 0.8	
LSD-25 (10^{-4} *M*)	53.5 ± 2.8 $P > 0.05$ (3)	10.7 ± 0.5	
LSD-25 (10^{-4} *M*) plus acorus oil (2×10^{-3})	42.5 ± 1.2 $P < 0.001$ (3)	37.3 ± 1.3	$P_i > 0.02$[c]
5-HT (10^{-3} *M*)	40.2 ± 0.3 $P < 0.001$ (4)	27.3 ± 0.8	
5-HT (10^{-3} *M*) plus acorus oil (2×10^{-3})	18.3 ± 2.7 $P < 0.001$ (4)	67.2 ± 2.5	$P_i < 0.001$

[a] Figures within parentheses show the number of experiments in each study.
[b] Probability (*P*) calculated by *t* test.
[c] Probability (P_i) calculated by *t* test due to the influence of LSD-25 and 5-HT on acorus oil.

The influence of LSD-25 (10^{-4} *M*) and 5-HT (10^{-3} *M*) on the respiration, with or without acorus oil (1:2,000), has been recorded in Table II. It will be seen from this Table that the inhibition produced by LSD-25 is of very little significance. It was, however, interesting to note that the addition of LSD-25 to the Warburg vessel containing acorus oil slightly reduced the inhibitive action of the oil; 5-HT, on the other hand, potentiated the action of oil considerably, even though it had individually inhibitory action on the respiration of brain.

It has been postulated that mental aberration induced by LSD-25 might be due to a metabolic antagonism between LSD-25 and 5-HT (8). Starbuck and Heim (9) have reported that LSD-25 has no effect on chlorpromazine while 5-HT partially inhibited the action of chlorpromazine *in vitro*. From our observations it is evident that LSD-25 partially reduced the inhibition caused by acorus oil and is in agreement with *in vivo* results reported by Dandiya, *et al.* (4). Potentiation caused by 5-HT *in vitro* on the inhibiting action of acorus oil lends further support to the suggested mechanism *in vivo*.

SUMMARY

1. The effect on the rate of respiration of the rat brain homogenate with various concentrations of the steam volatile oil of *Acorus calamus* L. has been studied by manometric technique.

2. Acorus oil was found to inhibit the oxygen uptake of brain tissue to a varying degree and ED_{50} was 1:1,980.

3. In the presence of glucose, sodium glutamate, and sodium succinate, acorus oil exhibited 43.1, 34.1, and 6.7 % inhibition, respectively.

4. LSD-25 reduced the inhibitory action of acorus oil while there was potentiation with 5-HT.

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