Technical Papers

Selective Inhibition of Brain Respirations by Benadryl[1]

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The following is a preliminary account of some experiments that form part of a larger program to examine the mode of action of antihistamines on the nervous system. It is becoming increasingly clear that the so-called antihistamines have effects in addition to those concerned with blocking the actions of histamine. Nerve cells appear to be especially obvious sites of action for the antihistaminic agents. Numerous investigators have called attention to the drowsiness, the hyperexcitability, the tremors, and the convulsions that may follow the administration of various doses of these compounds (*4*, *5*). The local anesthetic action, as well as the ability of the antihistamines to block nerve impulses in frog nerve fibers, has been reported (*1*, *2*, *6*).

The mechanism by which these compounds produce their effects on nerve cells is quite unknown. It is obvious that the metabolic actions of the antihistamines will have to be examined in order to supply a foundation upon which theories of the mechanism of action may be based. Thus far, biochemical studies of the antihistamines are few, although recently the inhibition by pyribenzamine of glucose and pyruvate oxidation and of anaerobic glycolysis in mouse brain homogenates has been reported by Hubbard and Goldbaum (*3*). The experiments to be reported here will demonstrate an interesting selective inhibitory effect of benadryl hydrochloride on the respiration of slices of rat cerebral cortex in a medium containing *l*-glutamate, in contrast to the respiration in a glucose medium.

The selective effect is summarized in Fig. 1. The oxygen consumption of slices respiring in a Krebs-Ringer phosphate solution (pH 7.35) with glucose and *l*-glutamate as substrates is shown in Curves 2 (glucose) and 3 (glutamate). The oxygen consumption with no added substrate (endogenous) is given in Curve 5. Each of these curves is the curve of best fit (by inspection) through the experimental points. Each experimental point represents the mean of duplicate readings. To avoid confusion the experimental points have not been included. The absence of effect of benadryl hydrochloride (0.0018 molar) on the slices respiring in a glucose medium is shown in Curve 1. It is clear that the addition of the benadryl at the 60-min point (*A*) produced no decrease in the rate of oxygen consumption. In contrast, the same concentration of benadryl hydrochloride completely blocked the oxygen consumption of the slices respiring in *l*-glutamate (Curve 4). This differentiation between the respiration in glucose and in *l*-glutamate was obtained in 17 experiments. The selectivity was especially striking when the benadryl hydrochloride was used in the concentration range of 0.0004–0.0018 molar. Concentrations above 0.0018 molar caused some inhibition of the glucose respiration. It is especially noteworthy that the endogenous respiration behaved in response to benadryl hydrochloride, not like respiration in glucose, but like respiration in *l*-glutamate (Curve 6). Results similar to the above were obtained also with pyribenzamine hydrochloride and with histadyl, but a thorough survey of the antihistamines and related substances remains to be done.

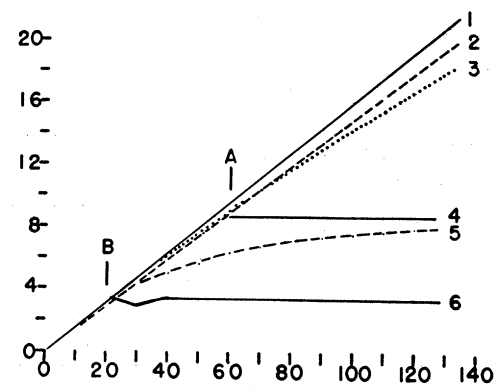

FIG. 1. The action of benadryl hydrochloride on the respiration of rat cerebral slices. Oxygen consumption (μl/mg dry wt) is plotted against time (min). Concentration of solutions: benadryl hydrochloride, 0.0018 molar; glucose, 0.011 molar; *l*-glutamate, 0.011 molar. Final volume, 0.9 ml; temperature, 38° C; pH, 7.35; gas phase, oxygen. Curve 1, glucose with benadryl hydrochloride added at 60 min (*A*); 2, glucose control; 3, glutamate control; 4, glutamate with benadryl hydrochloride added at 60 min (*A*); 5, endogenous respiration; 6, endogenous respiration, benadryl hydrochloride added at 20 min (*B*).

This differentiation of glucose and *l*-glutamate respiration was not obtained with certain other substances, which also depress the oxygen consumption of cortical slices. For example, *n*-amyl carbamate, *dl*-amidone, and morphine did not show the selective action of benadryl, the same inhibition being obtained in both glucose and *l*-glutamate media at all effective concentrations of these substances. The outcome of adding *n*-amyl carbamate (0.009 molar) is shown in Fig. 2.

It is of interest to inquire whether the inhibitory action of benadryl hydrochloride on brain slices respiring in *l*-glutamate can be reversed by means of histamine.

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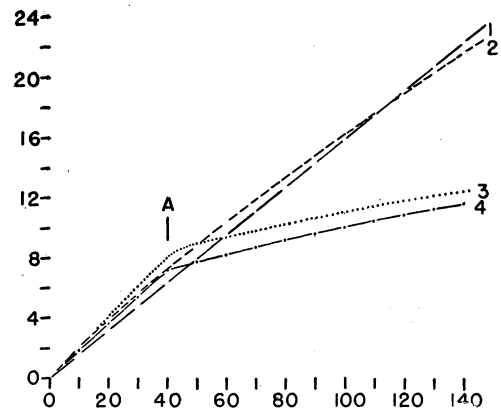

FIG. 2. The action of *n*-amyl carbamate. Solutions same as in FIG. 1, except for *n*-amyl carbamate (0.009 molar). Curve 1, glucose control; 2, glutamate control; 3, glucose with amyl carbamate added at 40 min (*A*); 4, glutamate with amyl carbamate added at 40 min (*A*).

That this cannot be done is illustrated in Fig. 3. Histamine dihydrochloride (pH adjusted to 7.35) at a concentration of 0.03 molar did not influence the rate of oxygen consumption of brain slices respiring in glutamate (Curve 2). Neither did histamine dihydrochloride prevent the inhibition of respiration by benadryl hydrochloride (Curve 4).

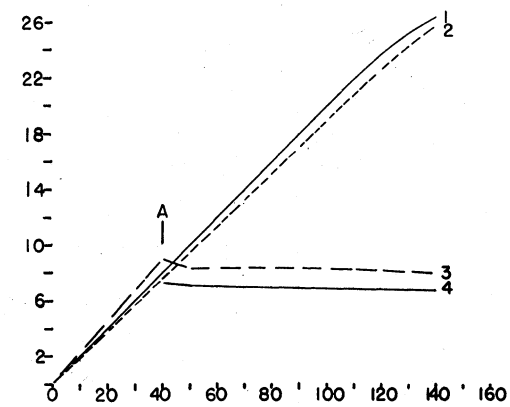

FIG. 3. Failure of histamine dihydrochloride to reverse benadryl effect. Solutions and conditions same as Fig. 1 except for histamine dihydrochloride (0.03 molar). Curve 1, glutamate control; 2, glutamate and histamine dihydrochloride; 3, glutamate with benadryl hydrochloride added at 40 min (*A*); 4, glutamate and histamine dihydrochloride with benadryl hydrochloride added at 40 min (*A*).

An analysis of the mechanism by which benadryl produces this selective inhibition of respiration will have to await the outcome of experiments now in progress. Obviously, selective alterations in permeability produced by the benadryl must be considered as a possible mode of action. The inhibition of respiration in experiments with *l*-glutamate and without added substrate makes the permeability concept unlikely, but experiments with brain homogenates, now in progress, should settle this point. If permeability is ruled out, the possibility exists that some metabolic step in the oxidation of *l*-glutamate is interfered with. Experiments with various metabolites linked to glutamic acid should prove useful in elucidating this point. All these matters will be discussed in a later report after the results of current experiments become available.

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Ammoniated Dentifrices and Hamster Caries: The Effect of Ingestion

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Experimental dental caries has been studied extensively in the Syrian hamster. Arnold (*1*) and Shafer (*6*) have shown that this animal is susceptible to dental caries when placed on a high-carbohydrate diet. Keyes (*4*) believes the carious process in hamsters is fundamentally the same as that observed in human beings.

Recently, ammoniated products have been proposed as an aid in controlling dental caries. Studies *in vitro* seem to indicate that an inhibitory effect on dental caries is possible. There has been very little actual testing of the effect of these products on caries in persons or in animals. Kesel (*2*) reported encouraging results from the use of a dentifrice and mouth rinse containing ammonia, but stated that further studies are necessary for conclusive results. Stephan (*7*) has shown that the application of urea in 40%–50% concentrations overcomes the effect of carbohydrate in lowering the pH of the dental plaque material. Keyes (*5*) studied the effect of weekly topical applications of a 50% urea solution on hamster teeth and found no apparent beneficial results.

It was thought desirable to devise an experiment in which the effect of the ammoniated compounds would be evaluated in regard to caries activity in the hamster. The effect of daily brushing with nonammoniated and ammoniated dentifrices, as well as the effect of ingestion, was studied. This report considers solely the effect of ingestion of dibasic ammonium phosphate and urea on caries incidence in the Syrian hamster.

The hamsters used were from an inbred colony. The animals were weaned at 21 days and placed on a diet of Purina Fox Checkers (with a weekly supplement of fresh carrots and hamburger meat), until they were approximately 30 days of age. The animals were separated into the experimental groups by sex and littermates.

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