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A PERIPHERAL ACTION OF SODIUM SALICYLATE

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Sodium salicylate and related compounds are widely used for their antipyretic properties. It has been suggested that the antipyresis is due to an effect on the hypothalamus, leading to increased heat loss (Barbour, 1926; Guerra and Barbour, 1943). This concept, however, does not specify what effects salicylate would be expected to have on metabolic rate.

The observation of Reid (1952), when studying the changes in acid-base balance, that sodium salicylate caused a marked increase in the O_2 consumption of the rabbit, was surprising in view of the antipyretic activity of the drug. The question arose, whether such an increase occurred in man with therapeutic doses of sodium salicylate. Denis and Means (1916) appear to have been the first to observe the effect clinically, but they did not attach any significance to it; Cochran (1952), however, clearly demonstrated that salicylate is a powerful metabolic stimulant in man.

The present study was to determine whether metabolic stimulation by sodium salicylate occurs only in the whole animal, or if it is also demonstrable in isolated tissue.

METHODS

The O_2 consumption of liver slices from CBA mice was determined by the direct method in Warburg respirometers at 37° C., according to the procedure of Dixon (1951). Glucose-phosphate-Ringer of *p*H 7.40 was used as medium. It was dispensed from double strength stock solutions and finally made up to volume in the Warburg flasks with water for the control tissues, or with a solution of sodium salicylate for the treated tissues.

Sufficient slices were obtained from each mouse to determine in duplicate the control rate of O_2 consumption, in c.mm. O_2 at N.T.P./hr./mg. dry weight of tissue (Q_{O_2}), and that in the presence of sodium salicylate.

Preliminary observations were made from a concentration of sodium salicylate of 10^{-5}M upwards. Systematic observations were then made at eight concentrations found to affect the Q_{O_2} of the liver slices; at each of these concentrations liver slices from sixteen mice, eight of each sex, were studied.

The duration of the runs was 30 min.; readings were taken at 15-min. intervals.

RESULTS

For each animal, the difference between the mean control and treated rates of O_2 consumption ($\triangle Q_{O_2}$) was obtained by subtracting the Q_{O_2} of the salicylate treated tissue from that of the control. Therefore increased rates of O_2 uptake appear as positive quantities and decreased rates as negative.

The mean response at each concentration is presented in Table I. A slight but definite increase in the rate of O_2 consumption was found at 3.5×10^{-4}M-sodium salicylate: this increase became progressively greater, and reached its maximum observed value at 2×10^{-3}M. From 3×10^{-3}M to 7.5×10^{-3}M the increase in rate of O_2 uptake diminished, giving way by 5×10^{-3}M to a decrease. At 2×10^{-3}M the mean $\triangle Q_{O_2}$ was +1.98; since the mean control Q_{O_2} was −9.10, this corresponds to a mean increase of 22% in the rate of O_2 consumption.

TABLE I

MEAN DIFFERENCES IN RATE OF O_2 UPTAKE BETWEEN CONTROL AND SALICYLATE TREATED LIVER SLICES

$\triangle Q_{O_2} = Q_{O_2}$ (control tissue) — Q_{O_2} (salicylate treated tissue). The last column shows the probability, according to the t-test, regarding the significance of the differences of the means from zero.

Molar Concn. of Sodium Salicylate	Mean $\triangle Q_{O_2}$ ± S.E.	P
3.5×10^{-4}	+0·41 ±0·08	0·001
5×10^{-4}	+0·68 ±0·14	0·001
1×10^{-3}	+1·36 ±0·14	<0·001
2×10^{-3}	+1·98 ±0·36	0·001
3×10^{-3}	+1·34 ±0·29	0·001
4×10^{-3}	+0·59 ±0·21	0·02
5×10^{-3}	−0·88 ±0·28	0·01
7.5×10^{-3}	−3·55 ±0·34	0·01

An indication of the precision of the data may be of interest. The means and standard deviations of the arithmetic differences between duplicate Q_{O_2} determinations were:—control tissues 0.31 ± 0.22, salicylate-treated tissues 0.37 ± 0.29. The grand mean control Q_{O_2} was -9.10 and the standard deviation ± 0.96.

The effects of sodium salicylate on the rate of O_2 consumption of mouse liver slices were therefore a simple stimulation between the concentrations

3.5×10^{-4}M and 2×10^{-3}M; at 3×10^{-3}M and 4×10^{-3}M an increasing depression was superimposed on the stimulation; and at 5×10^{-3}M and 7.5×10^{-3}M the depression predominated. No significant sex differences were found in the response of the liver slices to salicylate.

The relation between concentration of sodium salicylate and $\triangle Q_{O_2}$ is shown in Fig. 1. To establish the usual relation between response and logarithm of drug concentration, it is necessary to

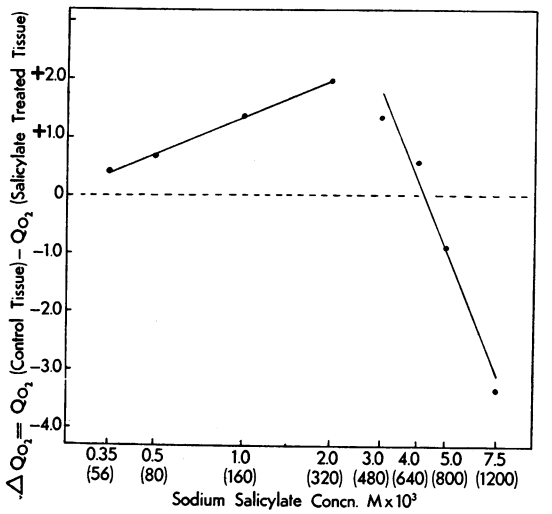

FIG. 1.—The relation between concn. of sodium salicylate and effect on O_2 uptake of mouse liver slices at 37° C., in glucose-phosphate-Ringer medium of *p*H 7.40. Abscissa: logarithmic scale of concn. M × 10³; in parentheses μg./ml. Ordinate: rate of O_2 consumption of control tissues less that of salicylate treated tissues ($\triangle Q_{O_2}$), in c.mm. O_2/hr./mg. dry weight of tissue. The points represent the mean differences found at each concn. The lines are drawn in accordance with the regression equations of $\triangle Q_{O_2}$ on log concn.

divide the data into two parts. Over the lower concentrations, up to 2×10^{-3}M, the mean $\triangle Q_{O_2}$ for each concentration bears a linear relation to the log of the concentration; the regression equation of $\triangle Q_{O_2}$ on log concentration and the analysis of variance are shown in Table II. (It was found convenient in the analysis of the data to use μg./ml. as the unit of concentration, rather than molar units.) The regression equation satisfactorily accounts for the relation between the magnitude of the effect and drug concentration.

Over the second part of the range of concentrations used—from 3×10^{-3}M—the relation between mean $\triangle Q_{O_2}$ and log concentration is not so strictly linear; inspection of Fig. 1 shows that the first mean $\triangle Q_{O_2}$ of this group, at 3×10^{-3}M, is somewhat out of line with the succeeding three. An explanation of this will be considered below;

TABLE II

THE REGRESSION EQUATIONS OF RESPONSE ON LOG CONCN. OVER THE CONCENTRATION RANGES (i) 3.5×10^{-4} TO 2×10^{-3}M AND (ii) 3×10^{-3} TO 7.5×10^{-3}M

$\triangle Q_{O_2} = Q_{O_2}$ (control tissue) $- Q_{O_2}$ (salicylate treated tissue). For simplicity, $x = \log_{10}$ concn. in μg./ml. The significance of the regression equations is indicated in the analyses of variance.

Concn. range of sodium salicylate	3.5×10^{-4} to 2×10^{-3} M (56 to 320 μg./ml.)			3×10^{-3} to 7.5×10^{-3} M (480 to 1,200 μg./ml.)		
Regression equation where $y = \triangle Q_{O_2}$ and $x = \log_{10}$ sodium salicylate concn. in μg./ml.	$y = 2.11x - 3.3$			$y = 35.65 - 12.65x$		
Analysis of Variance	Sum of Squares	d.f.	Mean Squares	Sum of Squares	d.f.	Mean Squares
Variance between concentrations:						
Due to regression equation	23.97	1	23.97	216.48	1	216.48
Deviation from regression	0.03	2	0.015	6.95	2	3.48
Variance within concentrations	40.87	60	0.68	77.56	60	1.29
Total Variance	64.87			300.99		

in the meantime it will be seen that a second regression equation was derived—presented in Table II—which establishes a significant relation between $\triangle Q_{O_2}$ and concentration of sodium salicylate.

It may seem feasible to determine the concentration at which the rate of O_2 consumption would be maximal—the optimal concentration for the stimulation—by solving the regression equations as a pair of simultaneous equations. Such a step is not generally permissible, and there is a specific reason for not doing so in the present instance. A diphasic concentration-response relation—that is, one which may be resolved into only two components—is theoretically improbable for the present data. Representation of the results in terms of two components is an approximation, since the condition is implied that the minimal concentration of maximal response for the stimulation (S) is the same as the threshold concentration for the depression (D). While this is possible, it is a special case. The general alternatives are (1) that S<D, when there would be, between the ranges of increasing stimulation and increasing depression, a range of concentrations within which the response would be maximal and constant; or (2) S>D—that is, the stimulation has not reached its zenith when the depression begins—in which case the concentration-response relation would consist of three components in all, the present second component being represented by two, one corresponding to simultaneously increasing stimulation and depression, the other to maximal stimulation and further depression. The latter alternative seems

applicable to the present results, and affords an explanation of the out-of-line point in Fig. 1.

In conclusion, it is not justifiable to estimate from the present data, by extrapolation, the concentration of sodium salicylate at which the rate of O_2 consumption would be maximal. Nevertheless, it is clear that this concentration lies between 2×10^{-3}M and 3×10^{-3}M.

Discussion

The only previous study of the effect of salicylate on tissue respiration appears to be that of Lutwak-Mann (1942), who reported depression at concentrations of 10^{-2}M and upwards.

The present investigation shows that sodium salicylate has two effects on the respiratory rate of mouse liver slices—stimulation succeeded, at higher concentrations, by depression. It cannot be assumed that these effects are universal, occurring in all tissues, but, following the precedent of other drugs exhibiting similar actions, it is probable that they are not confined to the liver. The stimulation is not peculiar to the species used, since salicylate is a metabolic stimulant in the intact rabbit (Reid, 1952) and in man (Cochran, 1952).

The stimulation is of more immediate interest than the depression, since it occurs at therapeutic drug concentrations, and because, unlike the depression, it is an unusual and apparently paradoxical action. Furthermore, the present study shows that this action is of peripheral, and not of central, origin.

In the past this type of effect has been particularly associated with 2:4-dinitrophenol (DNP) and certain of its derivatives, and the present results suggest that salicylate merits classification with this group of compounds. Stimulation of tissue respiration is not the only common factor in the pharmacodynamics of salicylate and DNP. DNP is glycogenolytic (Magne, Mayer, and Plantefol, 1933b); so is salicylate (Lutwak-Mann, 1942; Sproull, 1954). Both drugs stimulate respiratory movement out of proportion to the pulse rate; both are diaphoretic; both increase nitrogen excretion; body weight falls after repeated administration, and polyuria follows the withdrawal of both (Magne *et al.*, 1933a and b; Reid, Watson, and Sproull, 1950). McGuigan and Higgins (1935) caused fever in dogs by the administration of salicylate, and pyrexia occurs in non-allergic salicylate poisoning.

Emphasis has been placed on the similarity of salicylate and DNP, because the comparison furnishes a background for the interpretation of the present findings.

Since the stimulating concentrations are of the same order of magnitude as therapeutic plasma concentrations, metabolic stimulation by salicylate may be a factor in the therapeutic activity of the drug, or it may be a side-effect; at the moment neither possibility can be eliminated. The full importance of the present results cannot be assessed until evidence on this question is obtained. However, it is evident that sodium salicylate has the salient pharmacological properties of 2:4-dinitrophenol.

Summary

1. Rates of O_2 consumption of mouse liver slices in glucose-phosphate-Ringer at 37° C. were determined by the Warburg direct method, in the presence of graded concentrations of sodium salicylate, from 3.5×10^{-4}M to 7.5×10^{-3}M.

2. Progressively increasing respiratory rates were found from 3.5×10^{-4}M to 2×10^{-3}M-sodium salicylate; the maximum observed mean value, which corresponded to 22% more than the mean control Q_{O_2}, was found at 2×10^{-3}M.

3. From 3×10^{-3}M to 7.5×10^{-3}M-sodium salicylate the mean Q_{O_2} of the treated tissues fell, and became less than that of the controls by 5×10^{-3}M.

4. These results are briefly discussed, and attention is drawn to the numerous pharmacological properties shared by sodium salicylate and 2:4-dinitrophenol.

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