Brit. J. Pharmacol. (1959), **14**, 401.

THE ACTION OF NITROPHENOLS ON THE PULMONARY VENTILATION OF RATS

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(RECEIVED MAY 25, 1959)

The ventilatory effects of the three mononitrophenols and six dinitrophenols have been examined in anaesthetized rats. The minute volume of ventilation increased in all the test groups, the increase reaching the 99% significance level with seven compounds ($P<0.01$), the 95% level with 3-nitrophenol ($P<0.05$), and the 90% level with 3,5-dinitrophenol ($P<0.10$). The effects of 4-nitrophenol, 3,4-dinitrophenol, 3-nitrophenol, and 2,5-dinitrophenol, in increasing carbon dioxide output relative to oxygen consumption, are not explicable on the basis of simple hyperventilation, and are attributed to a metabolic effect at the cellular level. The potency of the nitrophenols in stimulating respiration is related to their structure, nitro groups being most effective in the *ortho* position and least effective in the *meta* position, and 2,4-dinitrophenol being the most powerful respiratory stimulant of the group. 2,6-Dinitrophenol does not conform to this generalization; one unique feature of its structure is indicated, as a possible explanation for the discrepancy. The gradation of potency of the nitrophenols (except 2,6-dinitrophenol) parallels the gradation of acidic properties in the group; the more strongly acid compounds stimulate respiration more powerfully. This is not a direct effect on blood *p*H, since the compounds were administered in neutral or slightly alkaline solution. Methaemoglobin formation was found to occur with 2,5-dinitrophenol, and to a smaller inconstant extent with three other compounds. Further work is suggested, to explore whether peripheral-acting ventilatory stimulation by 2,4-dinitrophenol is necessarily associated with peripheral metabolic enhancement, or whether the two effects can be dissociated.

The three mononitrophenols and six dinitrophenols have been compared by Cameron (1958) in respect of their effects on oxygen consumption of the rat. She found that 4-nitrophenol and 3,4-dinitrophenol increase carbon dioxide output without affecting oxygen uptake, and that 3-nitrophenol and 2,5-dinitrophenol depress oxygen consumption without affecting carbon dioxide output. While such relative stimulation of carbon dioxide output could be due to deviation from normal metabolic pathways at the cellular level, it might also be due to hyperventilation, and would in any case be modified by a change in the respiratory minute volume. To decide between these two possibilities, a study has been undertaken of the effects on ventilatory rate and depth of all nine of the compounds, including 2,4-dinitrophenol, the only true metabolic stimulant in the group.

METHOD

A pneumotachograph (Lilly, 1950) was constructed using the manometer of Greer (1958) and a low-inertia integrating motor. This was set up to record ventilatory volume over 53 sec. periods and respiratory rate over 30 sec. periods, and to allow monitoring on an oscilloscope of the respiratory airflow pattern (Silverman and Whittenberger, 1950).

Twelve anaesthetized tracheotomized rats could be studied at a time, allowing the ventilation of each to be measured four times/hr.

Wistar albino rats were used, in the age range 3 to 5 months. The mean weights were 270 g. (males) and 190 g. (females) with coefficient of variation 15%.

For anaesthesia, 16% ethanol by volume was given by stomach tube at an initial rate of 4.5 ml./100 g., and supplemented as necessary until preparations were obtained which would remain satisfactorily anaesthetized for the 2½ hr. period of the experiment.

The nine compounds were made up with sodium hydroxide in neutral or slightly alkaline solution

(Table III) and were administered in doses up to the maximum tolerable level as determined by Cameron (1958). A result was rejected if the three values to be averaged differed by more than 50%, if the animal died or needed more anaesthetic during the experimental period, or if respiration as monitored by the oscilloscope was gasping in character, or showed persistent irremediable airway obstruction.

Experimental Design.—After control observations over 1 hr., each animal received an intraperitoneal injection of normal saline or of a test compound; respiratory measurements were then continued for 1.5 hr. The first three observations of minute volume in the test period were averaged, and compared with the average of the last three control period observations, results being expressed as the ratio, average minute volume of respiration in test period/average minute volume of respiration in control period. A histogram of the frequency distribution of this ratio for 66 control animals showed positive skewness so the logarithms of the ratios were used for a comparison of each test group (9 to 21 animals) with the control group, using Cochran's modification of Student's t-test (Snedecor, 1956).

Results

The effects on respiratory minute volume are shown in Table I. Ventilation in the control period averaged 125 ml./min., with a mean rate of 78 respirations in each 30 sec. period.

TABLE I

EFFECT OF NITROPHENOLS ON MINUTE VOLUME OF RESPIRATION OF WISTAR ALBINO RATS ANAESTHETIZED WITH ETHANOL

Compound	Dose Range (mg.)	No. of Rats	% Increase in Respiration Compared with Control Period. Mean	with S.E.	t	P.
Control group ..	—	66	3·3	1·6	—	—
2,4-dinitrophenol	1–4	9	60	16	4·20	< 0·01
2,3-dinitrophenol	10–15	10	47	6	8·04	< 0·001
2,5-dinitrophenol	7–25	21	32	8	3·96	< 0·001
2-nitrophenol ..	60–120	16	27	7	3·66	< 0·01
4-nitrophenol ..	7–12	19	24	4	4·52	< 0·001
3,4-dinitrophenol	2–12	21	23	7	3·06	< 0·01
2,6-dinitrophenol	1–6·5	17	21	5	3·24	< 0·01
3-nitrophenol ..	20–45	17	15	5	2·15	< 0·05
3,5-dinitrophenol	4–7	16	11	4	1·98	< 0·10

In the test period, ventilation increased with all the compounds. The increase was significant at the 99% confidence level in seven instances ($P<0.01$), at the 95% level in one and at the 90% level in one. The effects on respiratory rate and tidal volumes are set out in Table II; in seven instances both rate and depth of respiration were increased, but in two the increased minute volume was produced entirely by tachypnoea.

Blood samples were examined for methaemoglobinaemia, using a reversion spectroscope.

TABLE II

EFFECTS OF NITROPHENOLS ON RAT RESPIRATION IN TEST PERIOD COMPARED WITH CONTROL PERIOD

Compound	Increase in Rate (%)	Increase in Tidal Volume (%)
2,4-dinitrophenol	16	37
2,3-dinitrophenol	12	31
2,5-dinitrophenol	9	21
2-nitrophenol	31	−5
4-nitrophenol	2	21
3,4-dinitrophenol	11	10
2,6-dinitrophenol	5	15
3-nitrophenol	24	−7
3,5-dinitrophenol	1·5	9
Control group	2	1

Substantial methaemoglobin formation was found with 2,5-dinitrophenol, and smaller inconstant amounts were formed with 2-nitrophenol, 2,3-dinitrophenol and 3,4-dinitrophenol.

Discussion

The wire screen pneumotachograph was well suited for studies in the rat, since there were no moving parts or valves to impede respiration, and the resistance of the screen was very low (about 1 mm. of water). Continuous monitoring on an oscilloscope of the air-flow pattern (pneumotachogram) provided a sensitive check on the character of respiration, and rapidly detected airway obstruction.

Methaemoglobinaemia (Bodansky, 1951) is associated with normal arterial oxygen tension, and does not produce respiratory distress unless the amount of normal haemoglobin is markedly reduced. The experimental results for 2,5-dinitrophenol should be treated with reserve, but the other results are not thought to be materially affected by methaemoglobin formation.

The present results differ completely from the visual observations of Cameron (1958) on the apparent depth and rate of respiration in her experiments. This illustrates the necessity for quantitative measurements of ventilatory volume in work of this type.

The dose ranges used for the different compounds are equivalent in that they represent approximately equal fractions of the lethal dose. Inspection of Table I shows a relation between structure and degree of respiratory stimulation. A nitro group in the *ortho* position is more potent than one in the *para* position; and a *meta* substituted group is least effective. 2,6-Dinitrophenol fails to conform to this structure function pattern; it may be relevant that this compound is unique in the series in having side chains on both sides of the hydroxyl group. A similar gradation

from *ortho* through *para* to *meta* exists in respect of the acidic properties of the compounds, as shown in Table III. The compounds were administered in neutral or slightly alkaline solution (*p*H 6 to 9, Table III) so the increases in ventilation were not caused by hydrogen ion administration.

TABLE III

COMPARISON OF EFFECTS OF NITROPHENOLS ON RAT RESPIRATION WITH THEIR ACIDIC PROPERTIES

Concentration and *p*H of drug solutions administered are given. *p*KA values at 25° obtained from Pauling (1940).

Compound	Increase in Respiratory Volume (%)	*p*KA	Conc.	*p*H
2,4-dinitrophenol ..	60	4·00	1%	7·1
2,3-dinitrophenol ..	47	4·92	3%	7·0
2,5-dinitrophenol ..	32	5·15	1%	7·7
2-nitrophenol ..	27	7·12	3%	..
4-nitrophenol ..	24	7·02	1%	8·1
3,4-dinitrophenol ..	23	5·43	2%	7·4
2,6-dinitrophenol ..	21	3·70	1%	6·2
3-nitrophenol ..	15	8·00	1%	8·4
3,5-dinitrophenol ..	11	6·68	2%	7·6

If the excess liberation of carbon dioxide were caused by hyperventilation, it would affect all the compounds in proportion to their effect on ventilation. If this were the only effect of the group on carbon dioxide metabolism, increased carbon dioxide production would vary in strict proportion to the effect on ventilation. The results do not show such a correlation, and prove that hyperventilation cannot be the sole cause of the effect on carbon dioxide production. The complete absence of any correlation suggests that hyperventilation is not even an important factor, and that the carbon dioxide effect is due to an action on cell respiration.

There is evidence that 2,4-dinitrophenol produces at least some of its hyperventilatory effect by stimulation of peripheral receptors, probably in muscle (Ramsay, 1955, 1959). It is tempting to suggest that the other nitrophenols act in the same way. Since these compounds do not increase oxygen uptake, it would then follow that peripheral metabolic enhancement was not an essential step in the peripheral ventilatory stimulus. The present results cannot settle the question, which needs further experiment.

I am grateful to Dr. J. R. Greer, Institute of Physiology, Glasgow University, and Dr. D. H. Sproull, of this Unit, for helpful advice.

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