7. W. L. STAFFORD, in *Salicylates* (Eds. A. ST. J. DIXON, B. K. MARTIN, M. J. H. SMITH and P. N. WOOD), p. 75. Churchill, London (1963).
8. A. LINDENBAUM and J. SCHUBERT, *J. phys. Chem.* **60**, 1663 (1956).
9. P. K. SMITH, HELEN L. GLEASON, C. G. STOLL and S. ORGORZALEK, *J. Pharmac. exp. Ther.* **87**, 237 (1947).
10. W. H. C. WALKER, *J. Clin. Path.* **20**, 786 (1967).
11. P. TRINDER, *Biochem. J.* **57**, 301 (1954).
12. R. J. BARTHOLOMEW and A. M. DELANEY, *Proc. Aust. Ass. clin. Biochem.* **1**, 214 (1966).
13. D. LESTER, G. LOLLI and L. A. GREENBERG, *J. Pharmac. exp. Ther.* **87**, 329 (1947).

Biochemical Pharmacology, Vol. 17, pp. 156–158. Pergamon Press. 1968. Printed in Great Britain

The influence of cyclic 3′, 5′-AMP and theophylline on oxygen consumption of rats

(*Received* 7 *August* 1967; *accepted* 14 *September* 1967)

THERE is much evidence that glycogenolytic and lipolytic actions of catecholamines are mediated by the formation of cyclic 3′, 5′-AMP (adenosine-3′, 5′-monophosphate).[1–4] Calorigenic action of catecholamines, on the other hand, is probably the consequence of catecholamine induced glycogenolysis and lipolysis.[5–8] Cyclic 3′, 5′-AMP, therefore, should be expected to possess calorigenic action too. However, the influence of cyclic 3′, 5′-AMP on metabolic rate has not been investigated till now.

Cyclic 3′, 5′-AMP is inactivated by a phosphodiesterase.[9] Methylxanthines such as caffeine, theophylline or aminophylline, inhibit phosphodiesterase,[10,11] and thereby may cause accumulation of endogenous 3′, 5′-AMP.[12] Methylxanthines thus also should be able to increase metabolic rate. In fact, caffeine and aminophylline are said to augment oxygen consumption.[13–16] But these investigations were performed only in awake animals and men. Therefore it is unknown, whether the calorigenic responses in those experiments were due to a real increase of basal metabolism or to increased motility because of the centrally stimulating actions of methylxanthines.

Thus, it was necessary to investigate the influence of cyclic 3′, 5′-AMP (Sigma Chemical Company) and theophylline (Theophyllinum, DAB 6) on the oxygen consumption of rats which had been anesthetized with urethane (1·2 mg/kg). Anesthesia by urethane excludes motility, but does not diminish calorigenic action of catecholamines in rats.[17]

Adult male wistar rats with a weight from 260 to 340 g were used. Oxygen consumption and respiratory quotient (RQ) were measured with NOYON's Diaferometer (Kipp and Zonen, Delft). After three determinations of basal metabolic rate within 30 minutes the drugs were injected, and oxygen consumption and RQ were recorded continuously throughout 120 min. The increase of oxygen consumption over basal metabolic rate was integrated for each animal and served (expressed as ml O_2 per 100 g body wt.) as an index of calorigenic response. Ambient temperature throughout the experiments was 28°. Rectal temperature of the rats lay between 36° and 37°.

The results of our experiments with regard to oxygen consumption are compiled in Table 1. Basal values of oxygen consumption ranged between 1·9 and 2·1 ml/min/100 g. Due to physiologic variations of metabolic rate, there was a small integrated increase of oxygen consumption in control animals injected with saline, but mean oxygen consumption did not exceed more than 8 per cent over basal values. In preliminary experiments 3′, 5′-AMP, injected intraperitoneally (100 mg/kg), did not produce a calorigenic response. The same was true for the intravenous application of 3′, 5′-AMP (Table 1): Integrated augmentation of oxygen consumption did not differ significantly from control values, though the doses of 3′, 5′-AMP were high (50 and 100 mg/kg).

The failure of 3′, 5′-AMP to increase oxygen consumption could have been the consequence of a rapid destruction by phosphodiesterase. Therefore, in another series of experiments theophylline (6·6 mg/kg i.p.) was given 30 min before cyclic 3′, 5′-AMP. Yet there was no increase of oxygen consumption in this group either (Table 1).

It has been demonstrated, that extrinsic 3′, 5′-AMP does not permeate into the cells in amounts sufficient to elicit pharmacological responses.[18] Thus, our negative results with exogenous 3′, 5′-AMP cannot be taken as evidence that endogenous 3′, 5′-AMP is not able to augment metabolic rate either.

TABLE 1. INFLUENCE OF CYCLIC 3′, 5′-AMP AND THEOPHYLLINE ON OXYGEN CONSUMPTION OF ANESTHETIZED RATS

Treatment	Dose mg/kg	N	Integrated increase of oxygen consumption within 120 min ($\bar{x} \pm s_{\bar{x}}$) ml/100 g	P
Saline		8	13·7 ± 4·1	
3′5′-AMP	50 i.v.	5	12·0 ± 3·8	>0·05
3′5′-AMP	100 i.v.	5	20·5 ± 3·1	>0·05
Theophylline + 3′5′-AMP	6·6 i.p. 100 i.v.	6	11·1 ± 4·2	>0·05
Theophylline	6·6 i.p.	5	33·7 ± 9·6	<0·01
Theophylline	20 i.p.	5	50·5 ± 5·5	<0·01
Theophylline	60 i.p.	5	67·2 ± 11·5	<0·01

Evidence for a mediator function of cylic 3′, 5′-AMP for catecholamine-induced calorigenesis was provided by our experiments with theophylline. This drug caused dose-dependent increases of metabolic rate in all tests (Table 1). The increment of mean oxygen consumption over basal values in the 3 series with theophylline were 17, 37 and 36 per cent, respectively. Together with the calorigenic response, there was a small increase of rectal temperature (0·1–0·3°), while the respiratory quotient (basal values lay between 0·74 and 0·82) increased slightly only after the highest dose of theophylline.

The integrated calorigenic response to 60 mg/kg theophylline i.p. corresponded approximately to that caused by 0·3 mg/kg adrenaline i.p. in anesthetized rats.[17] The time course of calorigenic action was, however, different for theophylline and catecholamines. After theophylline maximal values of oxygen consumption were not reached before 75 min after injection, and metabolic rate remained then on a high level. Maximal calorigenic responses to adrenaline and noradrenaline, on the other hand, occurred already 30 min after application, and oxygen consumption thereafter quickly returned to basal values.[17] This development of theophylline action indirectly supports the hypothesis that theophylline exerts its calorigenic action by diminished destruction and subsequent gradual accumulation of endogenous 3′, 5′-AMP. Further experiments, however, are necessary to prove this hypothesis.

Institut für Pharmakologie OTFRIED STRUBELT
der Medizinischen Akademie Lübeck
24 *Lübeck, Ratzeburger Allee* 160, *Deutschland*

REFERENCES

1. E. W. SUTHERLAND and T. W. RALL, *Pharmac. Rev.* **12**, 265 (1960).
2. E. W. SUTHERLAND and G. A. ROBISON, *Pharmac. Rev.* **18**, 145 (1966).
3. B. WEISS, J. I. DAVIES and B. B. BRODIE, *Biochem. Pharmac.* **15**, 1553 (1966).
4. A. AULICH, K. STOCK and E. WESTERMANN, *Life Sci.* **6**, 929 (1967).
5. L. LUNDHOLM, *Acta physiol. scand.* **19**, *suppl.* 67, (1949).
6. N. SVEDMYR, *Acta physiol. scand.* **68**, *suppl.* 274 (1966).
7. J. HIMMS HAGEN and P. B. HAGEN, in *Actions of Hormones on Molecular Processes* (Eds. G. LITWACK and D. KRITCHEVSKY), p. 298. Wiley, New York (1964).
8. D. STEINBERG, *Pharmac. Rev.* **18**, 217 (1966).
9. E. W. SUTHERLAND and T. W. RALL, *J. biol. Chem.* **232**, 1077 (1958).
10. R. W. BUTCHER JR. and E. W. SUTHERLAND, *Pharmacologist* **1**, 63 (1959).

11. R. W. BUTCHER and E. W. SUTHERLAND, *J, biol Chem.* **237**, 1244 (1962).
12. S. HYNIE, G. KRISHNA and B. B. BRODIE, *J. Pharmac. exp. Ther.* **153**, 90 (1966).
13. H. L. HIGGINS and J. H. MEANS, *J. Pharmac. exp. Ther.* **7**, 1 (1915).
14. J. BOCK, in *Handbuch der experimentellen Pharmakologie* (Eds. A. HEFFTER and W. HEUBNER), II. Band, 1. Hälfte, p. 508. Springer, Berlin (1924).
15. J. HALDI, G. BACHMANN, C. ENSOR and W. WYNN, *J. Nutr.* **21**, 307 (1941).
16. I. STARR, C. J. GAMBLE, A. MARGOLIES, J. S. DONAL, N. JOSEPH and E. EAGLE, *J. clin. Invest.* **16**, 799 (1937).
17. O. STRUBELT, *Arzneimittel-Forsch.* in press.
18. G. A. ROBISON, R. W. BUTCHER, I. ØYE, H. E. MORGAN and E. W. SUTHERLAND, *Molec. Pharmac.* **1**, 168 (1965).

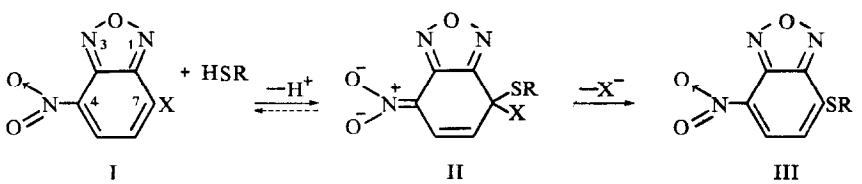
Biochemical Pharmacology, Vol. 17, pp. 158–161. Pergamon Press. 1968. Printed in Great Britain

4-Nitrobenzofurazans and 4-nitrobenzofuroxans: a new class of thiol-neutralising agents and potent inhibitors of nucleic acid synthesis in leucocytes

(*Received* 24 *July* 1967; *accepted* 30 *August* 1967)

KNOCK[1] has recently drawn attention to the need for new drugs to regulate the activity of nuclear protein thiol groups intimately concerned with gene control and neoplastic proliferation. We have found that certain benz-2,1,3-oxadiazoles (benzofurazans) and their 1-*N*-oxides (benzofuroxans) would seem to offer some promise as potential anti-leukaemic drugs or immunosuppressive agents as judged by the potent effects of 4-nitrobenzofurazan (NBFZ),† 4-nitrobenzofuroxan (NBFX) and some of their derivatives upon leucocyte metabolism *in vitro*.

NBFZ and NBFX are readily prepared by nitrating benzofurazan and benzofuroxan respectively.[2] Benzofurazan (m.p. 53)[3] is obtained from benzofuroxan (m.p. 73)[4] by opening the furoxan ring with hydroxylamine in KOH and steam distilling the dipotassium salt of *o*-benzoquinone dioxime which is formed as an intermediate. The preparation and *in vitro* pharmacological activity of over 60 related compounds will be reported elsewhere.[5]



RSH = Protein—thiol or glutathione

FIG. 1. Postulated drug action of 4-nitrobenzofurazans.

Fig. 1 summarises what we believe to be the mechanism of action of these drugs (for which evidence is provided below and elsewhere[5]). Molecules activated by a 4-nitro group (I) can add nucleophiles such as a mercaptide anion at position 7 forming a Meisenheimer-type complex (II), which may exist transiently (e.g. X = Cl) or may have an appreciable lifetime (e.g. X = SCN), subsequently decomposing to yield I or III. The proportion of unchanged drug (I) and irreversibly-formed end-product

† Abbreviations used: NBFZ = 4-nitrobenzofurazan; NBFX = 4-nitrobenzofuroxan; GSH = glutathione.