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A comparison of the effects of the optical isomers of isoproterenol on energy metabolism in a mouse sarcoma

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Summary. Disturbance to energy production in the S180 sarcoma (CB) by optical isomers of isoproterenol was assessed from altered adenine nucleotide levels at 1 h. The L-isomer almost halved the ATP level and lowered the energy charge significantly; the D-isomer was inactive. Dependence of tumor injury on cytochrome P-450 activity appears unlikely.
Key words. ATP; D-isoproterenol; energy charge; L-isoproterenol; S180 sarcoma (CB); tumor injury.

In 1957 it was reported that near-lethal doses of a number of hormones and drugs caused hemorrhage and necrosis in the murine S37 sarcoma[2]. Most of the active substances have now been examined[3-5] for their ability to interfere with energy production in the S180 sarcoma (ICRF), a variant of the tumor provided by the Imperial Cancer Research Fund, London, England. Vasopressin[3,5], hydralazine and L-isoproterenol[3-5] have been shown to be very effective in this respect at extremely low doses, although the catecholamines were not as active, even in more toxic amounts[3].

The question whether anti-tumor activity resides in one or in both isomeric forms of the catecholamines and isoproterenol has subsequently arisen; the present study has been restricted to examining the case of the sympathomimetic.

Materials and methods. D- and L-isomers of isoproterenol (3,4-dihydroxy-α-[(isopropylamino)methyl]-benzyl alcohol) D-bitartrate were purchased from Sigma London, England, and Sigma München, FRG, respectively. Enzymes and pyridine nucleotide cofactors were supplied by Boehringer, Mannheim, FRG.

Adult female mice (BALBc) were purchased from OLAC 1976 Ltd, England, and were implanted at 6–8 weeks of age with small pieces (3–5 mg wet wt) of the S180 sarcoma (CB) in the right groin by means of a trochar. The tumor was generously provided by Dr Dorothea Connell, Institute for Cancer Research, London SW3, England. Mice received s.c. injections of the isomeric forms of the sympathomimetic in 0.15 M pyrogen-free saline; control animals received vehicle alone (8 ml/kg). All mice were killed 1 h later. Tumors were freeze-stopped as described[6] within 8.7 ± 1.9 s. Enzymic methods[7,8] were employed to measure adenine nucleotides in neutralized (pH 6.5–7) $HClO_4$ extracts of the sarcoma[6].

Results. The S180 sarcoma (CB) showed dissimilarities with the ICRF variant used in previous studies[3-6,9]. For example, the CB tumor grew much faster, while histological examination revealed much more spontaneous background necrosis. In earlier work with the ICRF form, L-isoproterenol lowered the ATP level by 80% (dose, 2 mg/kg)[4] and by 71% (dose, 1 mg/kg)[5].

The table shows that the response to the L-isomer in this instance was not as marked, the recorded fall in the ATP level being only 45%. Nonetheless, the rise and fall seen in the levels of AMP and ATP respectively were highly significant. In contrast, the D-isomer was completely without effect, all measured parameters remaining substantially unaltered after treatment.

Discussion. The possibility that the changes in energy metabolism brought about by L-isoproterenol develop as a consequence of decreased blood flow through the tumor mass cannot be dismissed out of hand in the absence of direct measurements, but would nonetheless appear to be remote for the following reasons. Upset to energy production caused by the sympathomimetic in the S180 sarcoma (ICRF) can be largely prevented by pretreatment with indomethacin[9]; according to the above argument, protection conferred by the antiinflammatory agent would necessarily entail relative constancy of blood flow

The effects of L- and D-isoproterenol on the adenine nucleotide content of the S180 sarcoma (CB) in BALBc mice 1 h after an s.c. injection of 1 mg base/kg b.wt

Treatment	Metabolites, μmoles/g wet weight*			Energy charge[13]
	ATP	ADP	AMP	
L-Isomer	0.58 ± 0.22**	0.46 ± 0.08	0.43 ± 0.13**	0.54 ± 0.10**
D-isomer	1.07 ± 0.15	0.49 ± 0.04	0.10 ± 0.01	0.79 ± 0.02
Saline control	1.06 ± 0.13	0.48 ± 0.12	0.14 ± 0.07	0.78 ± 0.03

Each group comprised four tumors. Energy charge[13] = (ATP + ½ ADP) ÷ (ATP + ADP + AMP).
* Values represent means ± SD. ** $0.01 > p > 0.002$ (Student's t-test); comparisons made with saline control.

through the tumor. Noradrenaline consistently and dramatically lowered the blood flow through various tumors of both the rat[10,11] and the mouse[12], while in the same systems isoproterenol had either a much smaller effect[10,12] or none at all[11]. In contrast, study of the S180 sarcoma (ICRF) showed that interference with energy production caused by L-isoproterenol was much greater than that brought about by noradrenaline, falls in the energy charge[13] being 0.54 and 0.22 respectively[3] after 1 h. If the cutback in energy production resulted largely from decreases in blood flow, then the reverse situation would be expected.

The mechanism of interference of L-isoproterenol with energy metabolism in the sarcoma remains to be elucidated in detail; meanwhile the inhibitory effect of indomethacin pretreatment[9] points to involvement of a dioxygenase and participation of free radicals derived from oxygen[5]. The precise manner in which reactive oxygen species might arise is still a matter for speculation. When acting upon substrate in the presence of NADPH, cytochrome P-450 from rat liver microsomes can produce hydrogen peroxide[14] derived from superoxide[15]. Cytochrome P-450 has also been found in tumors of nonhepatic origin[16,17], but has not apparently been reported in any form of the S180 sarcoma. Moreover, the racemate of isoproterenol, given by the intravenous route in the dog[18,19] and in man[19], was either excreted unchanged or converted enzymically to 3-O-methyl derivatives possessing weak β-adrenergic blocking activity[19]. If the sympathomimetic were to encounter a similar metabolic fate in the mouse, then the chances of tumor injury arising from free radicals produced by cytochrome P-450 acting on the L-isomer would appear to be unlikely. Alternatively, the action of L-isoproterenol might be to increase the proportion of active oxygen species escaping from electron transport particles during oxidative phosphorylation[20].

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The effects of vitamin A nutritional status on glutathione levels and microsomal lipid peroxidation in rat lung[1]

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Summary. In vitamin A-deficient rats, the glutathione level in lung was diminished and microsomal lipid peroxidation much increased. In vitamin A-loaded animals, however, both were depressed below control. Thus vitamin A protection against lipid peroxidation is independent of glutathione.
Key words. Vitamin A; lipid peroxidation; glutathione; lung.

In a recent study[3] we found that peroxidation in rat liver microsomal lipids was inversely related to dietary intake of vitamin A, whereas microsomal cytochrome levels were depressed in both vitamin A-deficient and vitamin A-loaded animals, and certain microsomal metabolic activities were abnormal only in the vitamin A-deficient state. Apparently, the role of vitamin A is multifacetted. Since the lung is known to be especially vulnerable to environmental oxidants[4], we have undertaken another study relating to the effects of dietary vitamin A on the lung.

Materials and methods. The animals were prepared as described previously[3]: in brief, weanling Sprague-Dawley rats weighing initially 50 g were maintained for 90 days on diets providing either 5, 90 or 500 µg/day retinyl acetate. At the end of this period, the vitamin A-deficient group showed signs of moderate hypovitaminosis whilst the vitamin A-loaded group were not noticeably abnormal.

The rats were fasted overnight for 16 h, then lightly anesthetized with ether. Blood was collected from the orbital plexus and the animals were killed by cervical dislocation before removing the lungs. These organs were weighed, washed with ice-cold 0.15 M KCl–0.02 M Tris buffer, pH 7.4, and homogenized in 3 volumes of buffer. The 9000 × g supernatant and microsomal fractions were prepared by differential centrifugation of the resulting homogenate following the procedure of Tom and Montgomery[5]. The protein concentration of the subcellular fractions was measured by the method of Lowry[6]. Glutathione level in the 9000 × g supernatant fraction was determined according to Ellman[7]. Microsomal lipid peroxidation was estimated by the formation of thiobarbituric acid-reactive products in the presence of NADPH and ADP-Fe(III) complex[5].

The liver was also removed from some animals and a portion solubilized in boiling 30% KOH for 15 min. Vitamin A (retinol)