The influence of aromatic amines and folate on acid production by *Escherichia coli*

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Summary. Folinic acid, adrenaline and noradrenaline, substances known to stimulate oxygen consumption by *E. coli*, are shown in this experiment to do so by different mechanisms. This work demonstrates stimulation of acid production by folinic acid and inhibition by adrenaline and noradrenaline.

Folinic acid has stimulatory effects on several types of mammalian cells[2,3] which are similar to those produced by catecholamines. More recently it has been shown that folinic acid, noradrenaline and adrenaline all stimulate the oxygen consumption of bacteria[4]. The following is an investigation of the effects of these substances on bacterial glycolysis by measuring the rate of acid production by *Escherichia coli* in a glucose medium.

Materials and method. Glycolytic activity was assayed using a semi-automatic pH-stat assay system comprising a combined electrode type GK 2321C, a pH-meter type 26, a titrator TTT11 and a 0.25-ml-volume autoburette type ABU 1c (Radiometer, Copenhagen) and a potentiometric recorder (Smiths Servoscribe). The autoburette, designed to give a mechanical signal, was modified by the addition of a potentiometric device to give an electrical signal which could be fed into the recorder. The assays were performed at 30 °C in a total volume of 2.0 ml. The solutions were continuously magnetically stirred. The burette end point was set at pH 7.0 and 16.8 mM CO_2-free sodium hydroxide was used as the titrant.

A single colony of *E. coli* (human intestinal, type not identified) was subcultured on a fresh blood agar plate. The culture was harvested and thrice washed with Krebs-Ringer[5] solution. After centrifuging at 2000 × g for 20 min, 0.1 ml of the bacterial pellet was placed in the cell of the pH-stat.

Drugs which inhibited acid production

Drug	Concentration (M)	Rate of acid production (compared with pre-drug rate)
Noradrenaline	2.9×10^{-5}	0.85
	2.9×10^{-4}	0.50
	5.8×10^{-4}	0.35
	1.0×10^{-3}	0.0
Adrenaline	5.8×10^{-5}	0.7
	2.9×10^{-4}	0.32
	1.0×10^{-3}	0.0
Phenylalanine	1.2×10^{-3}	0.9
	5.8×10^{-3}	0.7
Metaraminol	4.5×10^{-5}	1.0
	4.5×10^{-4}	0.2
	1.0×10^{-3}	0.0
Methoxamine	4.0×10^{-4}	0.8
	1.2×10^{-3}	0.75
Terbutaline	1.0×10^{-5}–1.5×10^{-3}	1.0
Isoprenaline	1.0×10^{-5}–1.5×10^{-3}	1.0
Salbutamol	1.0×10^{-5}–1.5×10^{-3}	1.0
Dopamine	1.0×10^{-5}–1.5×10^{-3}	1.0
Ephedrine	1.0×10^{-5}–1.5×10^{-3}	1.0
Amphetamine	1.0×10^{-5}–1.5×10^{-3}	1.0
Phenylethylamine	1.0×10^{-5}–1.5×10^{-3}	1.0
Sodium glutamate	1.0×10^{-5}–1.5×10^{-3}	1.0
Glutamine	1.0×10^{-5}–1.0×10^{-3}	1.0
Octopamine	1.0×10^{-5}–1.0×10^{-3}	1.0
Tyramine	1.0×10^{-5}–1.0×10^{-3}	1.0
Folinic acid	1.0×10^{-5}	1.2
	2.5×10^{-4}	2.1
Folic acid	2.0×10^{-5}–2.5×10^{-4}	1.0

Results and discussion. Folinic acid (Leucovorin) produced a stimulation of acid production, the peak being at a concentration of 2.5×10^{-4} M, when acid production was doubled. Folic acid (10^{-5}–10^{-3} M) produced no effect.

Of the amines tested only noradrenaline, adrenaline and meteraminol were capable of completely inhibiting acid production (usually at 10^{-3} M); these substances gave a dose-dependent inhibition at lower concentrations (table). Phenylalanine and methoxamine inhibited acid production to a lesser extent (20–30%) at concentrations of 5.8×10^{-3} M and 1.2×10^{-3} M, respectively. The table also shows a wide range of aromatic amines which did not effect acid production at concentrations of 10^{-5}–10^{-3} M. The list includes the β agonists terbutaline, isoprenaline and salbutamol.

The inhibitory effects of noradrenaline and adrenaline were not modified by the α blockers thymoxamine (1.8–7.2×10^{-4} M) and phentolamine (1.8×10^{-4} M) or by the β blockers propranolol and practolol (both in the range 1.0–8.0×10^{-5} M), although on 1 occasion 7.8×10^{-5} M propranolol produced some restoration of acid production in organisms exposed to 1.5×10^{-4} M noradrenaline.

Dibutyryl 3′,5′-cyclic AMP at concentrations of 1.25×10^{-4}–1×10^{-3} M on its own did not influence the rate of acid formation.

The results suggest that the stimulatory effects of folinic acid, adrenaline and noradrenaline on bacterial oxygen utilisation do not occur by a common mechanism, because whereas folinic acid stimulates acid production in this experiment, the catecholamines inhibit this process. The inhibitory actions of adrenaline and noradrenaline on acid production could represent a metabolic switch in bacteria to an increase in the rate of oxidative phosphorylation. Conversely the stimulatory actions of folinic acid (but not folic acid) on both oxygen uptake and acid production could be due to an acceleration of net glucose utilization by the cell.

The effects of adrenaline and noradrenaline on acid production cannot be analyzed by the α/β receptor theory. Blockade of α and β receptors has little or no effect on the inhibitory process. β and α agonists other than adrenaline, noradrenaline and metaraminol, such as terbutaline, salbutamol and methoxamine have little or no effect on acid production. The 3 active substances could be primarily acting intracellularly, but presumably not via 3′,5′-cyclic AMP because this substance has no effect on acid production when added to the pH-stat cell. In fact the only correlation for this grouping of drugs is with the previous work on oxygen consumption by *E. coli*[3] in which it was observed that adrenaline and noradrenaline stimulate respiration whereas isoprenaline, salbutamol, and other sympathomimetic drugs do not.

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