Effects of Drugs and Chemicals on Subcellular Organelles: Mitochondrial Contraction and Coronary Dilators[1] (36904)

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In a previous paper (1) it had been demonstrated that ATP (adenosine triphosphate) shrinking of osmotically swollen mitonchondria, reflects *in vivo* change in those mitochondria produced by drug and nerve stimulation. Stimulation of the vagus toward the heart in those experiments produced an increase in the shrinking of heart mitochondria taken from rabbits after hours of stimulation. A factor identified with this augmented effect of mitochondria shrinking was the catecholamines released by excitation of the vagus.

Vagal stimulation also produces a brief period of asystole during which coronary blood flow is diminished or absent. Such periods of reduced tissue oxygenation could act as a stimulus for release of adenosine which normally appears in coronary blood only after interrupted coronary flow (3). Thus a possible additional chemical mediator of the mitochondrial changes of vagal excitation could be adenosine or related nucleosides.

Adenosine has been proposed to be a physiologic regulator of coronary blood flow (3) and it is both coronary vasodilator and hypotensive at pharmacologic doses (4, 5). This suggested that adenosine and other coronary vasodilators and vagal stimulation be examined together to further elucidate the mechanisms of volume change produced in heart mitochondria.

Materials and Methods. A detailed description of the method for measuring mitochondrial volume changes can be found in an earlier communication (1). Briefly, heart mitochondria are isolated in a medium of 0.18 *M* KCl, 10 m*M* EDTA and 0.5% bovine serum albumin by centrifugation. These mitochondria (approximately 10 mg biuret protein in isolation medium) are added to 3.0 ml of 200 mOsm Na_2SO_4. A decrease in optical density at 520 nm is taken as evidence of swelling. After 20 min, 0.1 ml containing ATP (2 m*M*), $MnCl_2$ (3 m*M*) and $CaCl_2$ (3 m*M*) is added and the increase in OD (shrinking) is recorded over the next 20 min. The ratio of maximum shrinking/maximum swelling is then calculated and used as an indicator of *in vivo* effects of drugs and physiologic stimulation.

Experimental conditions. Male, white New Zealand rabbits were anesthetized with pentobarbital sodium (30 mg/kg) injected via the marginal ear vein. The left common carotid artery was cannulated and used to record blood pressure with a Statham P23 Db transducer. Measurements were recorded on a multichannel polygraph.

The vagi were isolated bilaterally and either the right or left cardiac vagus was stimulated at the level of the thyroid after both had been ligated and crushed centrally. Stimulation was for a 30 sec interval at 50 Hz, every 2.5 min for 2 hr and then the animals were sacrificed. A voltage was used which produced a nearly complete period of asystole (3–10 V). Infusion of drugs was made into the right external jugular vein at a rate of 0.1 ml/min.

Statistical comparisons of all results were made by single tail *t* test (6).

Results. Intermittent stimulation of the vagus for 2 hr produced a significant ($p < 0.01$) increase in mitochondrial volume changes when compared with sham stimulated anesthetized controls so that this time period was adapted for the entire study.

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TABLE I. Interaction Between Drugs and Vagus Stimulation on Mitochondrial Shrinking by ATP.[a]

	N	% Rise in OD after ATP[a]	Probability
Drugs, without vagus stimulation			
Control	4	45 ± 2	
Dipyridamole, 0.1 mg/kg, 2 injections	4	62 ± 3	<.01[b]
Adenosine, 0.5 μM/kg/min, infusion	4	41 ± 3	<.3[b]
Nitroglycerin, 5 μg/kg/min, infusion	4	85 ± 4	<.01[b]
Drugs, with vagus stimulation			
Vagus stimulation	4	67 ± 5	<.01[b]
Vagus and dipyridamole	4	97 ± 8	<.02[c]
Vagus and adenosine	4	84 ± 3	<.05[c]
Vagus and nitroglycerin	4	106 ± 8	<.01[c]
Vagus and adenosine 5 monophosphate, 0.5 μM/kg/min infusion	4	66 ± 2	<.8[c]
Vagus and inosine, 0.5 μM/kg/min, infusion	4	65 ± 3	<.7[c]
Drugs and vagus after phentolamine, 1 μg/kg/hr, 2 injections			
Vagus stimulation	4	42 ± 2	<.01[d]
Vagus and nitroglycerin	4	42 ± 3	<.01[d]
Vagus and adenosine	4	45 ± 6	<.01[d]
Vagus and dipyridamole	4	41 ± 4	<.01[d]

[a] Mean value ± SE for N experiments.
[b] Compares mean value with control mean value.
[c] Compares mean value with value for vagus stimulation alone.
[d] Compares mean value with value before phentolamine.

Three sets of experiments were performed beginning with the interaction between drugs and vagal stimulation. As seen in Table I, adenosine, dipyridamole and nitroglycerin synergized with vagal stimulation to increase the amplitude of ATP-induced, mitochondrial volume changes. The monophosphate of adenosine and the deaminated product of adenosine, inosine, did not influence the effect of vagal stimulation.

Each of the 3 interactive compounds was also studied in the absence of vagal stimulation. An infusion of 0.5 μM/kg/min of adenosine produced a slight fall in mean arterial pressure which was maintained at a steady state after the first 5 min. This infusion of adenosine did not affect mitochondrial volume change significantly ($p < 0.3$) as seen in Table I. Dipyridamole, 0.1 mg/kg was given by intravenous injection as a bolus at the start of each experiment and again after 1 hr. Each injection caused a sharp but transient fall in mean arterial pressure. Mitochondria of hearts taken from rabbits given 2 injections of dipyridamole showed greater ATP shrinkage ($p < 0.01$) than controls (Table I).

Nitroglycerin was infused at a rate of 5 μg/kg/min. At this rate of infusion, nitroglycerin caused a persistent fall in mean arterial blood pressure. Mitochondria from hearts exposed to nitroglycerin showed significantly greater ($p < 0.01$) ATP shrinkage than did controls (Table I).

In the final set of experiments, vague stimulation and drugs were studied in the presence of phentolamine. As shown in Table I, phentolamine prevented the synergism between vagal stimulation and adenosine, dipyridamole and nitroglycerin. Moreover, this drug also reduced the effect of vagal stimulation on ATP-induced, mitochondrial shrinking.

Discussion. These experiments have once again demonstrated that reversible volume changes in heart mitochondria as measured *in vitro,* reflect *in vivo* effects produced by drugs on these organelles.

Vagal stimulation, which results in an increased amplitude of swelling–contraction was synergized by adenosine, dipyridamole and nitroglycerin. Two of these substances, dipyridamole and nitroglycerin, produce therapeutically useful increases in coronary blood flow.

Vagal stimulation alone is associated with an increase in coronary flow in some species. Feigl (15) has shown in dogs that vagal stimulation causes coronary dilatation, independent of any chronotropic or inotropic effects which can be purely a parasympathetic effect. However vagal stimulation of an intensity sufficient to produce asystole can possibly also cause the release of endogenous adenosine.

The typical cardiovascular effects of adenosine include a negative chronotropic and inotropic effect (5), hypotension and coronary dilatation (4). When given as a single injection, the effects are brief owing to adenosine's rapid deamination to inosine by adenosine deaminase (8). However, infused intravenously, as is this investigation, adenosine was able to synergize the effect of vagal stimulation on mitochondrial swelling–contraction. The failure of adenosine alone to affect heart mitochondria in this way, probably reflects the action of erythrocyte deaminase, which reduced the amount of adenosine able to reach the myocardium.

Adenosine can also arise endogenously in heart tissue from the action of 5′-nucleotidase on AMP (9), AMP, however, failed to synergize the vagal stimulation which may indicate that rabbit myocardium is much more permeable to adenosine than its phosphorylated derivatives (10). Inosine, which arises from deamination of adenosine, is reported to be essentially devoid of vasodilatory activity (8) and it did not synergize the vagal stimulation.

Dipyridamole, a "nonnitrate" coronary vasodilator, has been shown to inactivate erythrocyte deaminase (11) and to increase some of the pharmacologic effects of adenosine (12). Thus its synergistic effect with vagal stimulation on mitochondrial swelling–contraction could be referable to a "sparing" effect on endogenous adenosine.

Nitroglycerin is a well-established coronary vasodilator and appears to act independently of neurohormonal influence (13). It has little effect on mitochondrial oxidative phosphorylation (14) and does not appear to act through release of adenosine (13). Nitroglycerin synergized with vagal stimulation to increase the amplitude of swelling–ATP contraction and also did the same when infused alone. Because the action of nitroglycerin on coronary vessels appears unrelated to adenosine, it would seem that the effect of coronary dilator drugs on heart mitochondria can also arise through more direct mechanisms without involving adenosine.

All the means employed in this investigation to increase mitochondrial swelling–contraction could be blocked with phentolamine. The drug possesses alpha adrenergic blocking activity and alpha adrenergic receptors have been demonstrated in the coronary vessels of dogs (15). Feigl (15) has shown that stellate ganglion stimulation leads to coronary vasoconstriction which can be blocked with the alpha adrenergic blocker, dibenzyline. Hence in a sense, blockade of alpha adrenergic receptors with phentolamine is like causing relative coronary dilatation. There is, however, evidence that phentolamine may also act by increasing release of norepinephrine (16). In the isolated perfused rabbit heart, phentolamine doubled the outflow of norepinephrine during sympathetic nerve stimulation (17). Our previous experiments with vagally induced mitochondrial swelling–contraction have shown that agents which deplete heart catecholamine also prevent this effect, and phentolamine may be acting by this mechanism.

Summary. The interaction between vagal stimulation and agents with coronary dilator activity was studied in heart mitochondria of rabbits. Vagal stimulation alone, increased the amplitude of mitochondrial swelling–contraction. The effect was synergized by adenosine, dipyridamole and nitroglycerin. Phentolamine prevented both the vagal effect and its synergistic effect with the coronary dilator drugs leaving mitochondrial swelling–contraction at control levels.

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