from alloxan-diabetic rats show diminished glucose uptake and responsiveness to insulin when compared with those from normal rats. Diaphragms incubated in a medium containing free fatty acids (FFA) show a metabolic pattern similar to that seen in diabetes, in which a high rate of FFA oxidation causes inhibition of glycolysis and hence of glucose uptake (Randle, Garland, Hales & Newsholme, 1963). Metformin (10 μg/ml) produced a significant increase in glucose uptake in the presence of insulin (100 μU/ml) by diaphragms from alloxan-diabetic rats and also by normal diaphragms incubated with sodium butyrate (25 mg/100 ml).

In normal muscle tissue the rate of glucose utilization is limited by membrane transport, whereas in diabetes transport is limited by the rate of intracellular glucose metabolism, which is impaired as described above. It is suggested that metformin increases the rate of intracellular glucose metabolism, thus enhancing membrane transport in the diabetic state but having no effect on transport in normal muscle.

TABLE 1. *Effect of metformin on glucose uptake by isolated rat diaphragm*

	Normal diaphragm								Diaphragm in sodium butyrate (25 mg/100 ml)		Diaphragm from alloxan-diabetic rat	
Insulin concentration (μU/ml)	0		100				1,000		100		100	
Metformin hydrochloride concentration (μg/ml)	0	10	0	10	0	100	0	10	0	10	0	10
Mean glucose uptake*	5·7	6·1	10·0	10·7	10·6	9·6	12·0	12·1	8·7	9·7	5·9	6·9
Mean percentage change ± S.E.M.†	+27±27		+8±6		−10±2		+3±8		+12±4		+27±12	
t	1·0		1·4		4·4		0·4		3·4		2·3	
Number of experiments	8		12		8		8		10		10	
Significance	N.S.		N.S.		$0{\cdot}01>P>0{\cdot}002$		N.S.		$0{\cdot}01P>>0{\cdot}002$		$0{\cdot}05>P>0{\cdot}02$	

* Results are expressed in mg glucose taken up per g fresh weight of tissue in 90 min.
† All comparisons are made on a "within rats" basis.
N.S.=Not significant.

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Evidence that the potentiation by neostigmine of constrictor responses of the rabbit ear artery is not due to anticholinesterase activity at adrenergic nerve endings

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The finding that anticholinesterases, including physostigmine and neostigmine, potentiate responses to electrical stimulation of adrenergic nerves has been used as evidence for the involvement of acetylcholine in such transmission at various sites

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(Burn & Rand, 1960; Burn & Malik, 1970). However, Herron, Mock & Wallace (1971) showed that potentiation by physostigmine of contractile responses of isolated rabbit ear and femoral arteries was not specific for electrical stimulation but occurred to a similar extent with responses to noradrenaline and histamine injected into the perfusate.

In the present experiments, isolated perfused rabbit central ear arteries were induced to contract by injections of noradrenaline and histamine and by trains of 60 square wave pulses of 10 msec duration, supramaximal voltage and frequency 1–10 Hz. Neostigmine was added to the perfusate in concentrations of 5, 10, 20, 40 and 80 mg/l.; the order of administration was randomized. Responses to noradrenaline, histamine and electrical stimulation were increased by neostigmine, the increase in response to all these stimuli being maximal and significant ($P<0.05$) at around 40 mg/l. of neostigmine. The absolute increase in response size was similar for noradrenaline, histamine and electrical stimulation (Fig. 1).

When sodium in the perfusate was replaced by potassium, the arteries no longer responded to electrical stimulation but responded to noradrenaline and histamine. These responses were not now potentiated by neostigmine. The potentiating actions of 5-hydroxytryptamine and of cooling are also antagonized by a high potassium perfusate (de la Lande, Cannell & Waterson, 1966; Glover & Wallace, 1969). It has been suggested that these sensitizing agents act by slightly

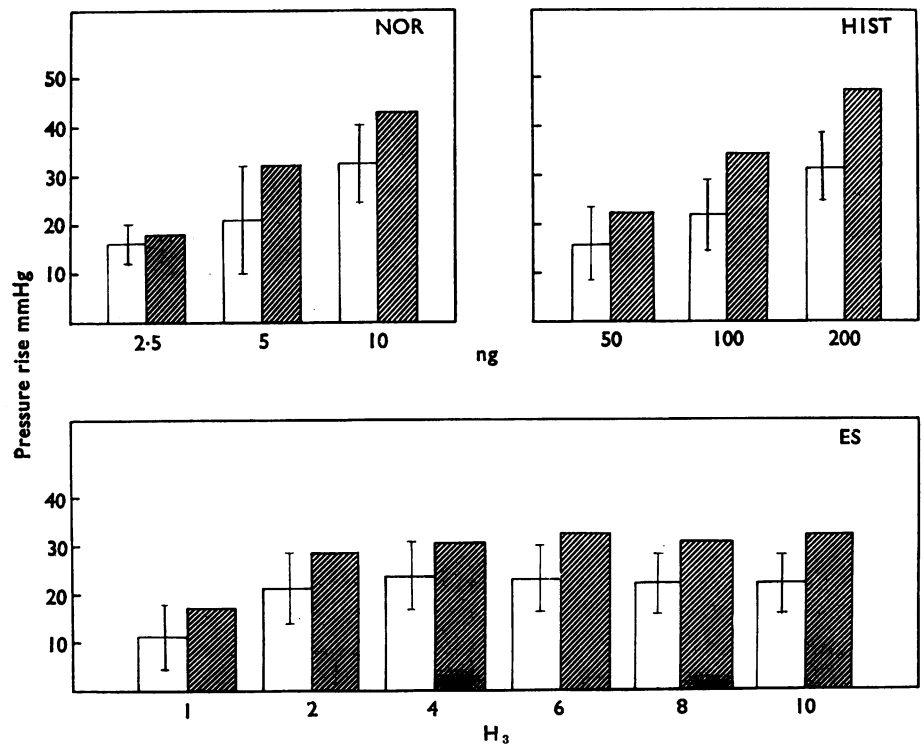

FIG. 1. Effect of neostigmine methyl sulphate (NEO) 40 mg/l. on responses (pressure rise, mmHg) to injected noradrenaline (NOR, 2·5, 5, 10 ng) and histamine (HIST, 50, 100, 200 ng) and to electrical stimulation (ES, 1, 2, 4, 6, 8, 10 Hz). Open rectangles: control results; hatched rectangles: NEO present. Mean results for 8 arteries; where the vertical bars do not overlap the top of the hatched rectangles, responses were significantly increased by NEO ($P<0.05$, multiple F test).

depolarizing smooth muscle cells; the anticholinesterases, physostigmine, and neostigmine, may have a similar action. This possible action should be borne in mind at any site where these anticholinesterases act as sensitizers.

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The action of propranolol on the dog heart

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Propranolol does not depress the isometric twitch of isolated skeletal muscle or isolated papillary muscle to electrical stimulation in concentrations associated with β-adrenoceptor blockade (Harry, Linden & Snow, 1971). Further evidence is presented here that propranolol in doses associated with β-adrenoreceptor blockade does not depress the myocardium of the intact heart of the dog.

Dogs were anaesthetized with chloralose and the chest opened in the mid-line. The heart was denervated by cutting the vagi in the neck and crushing both ansae subclaviae. The maximum rate of rise of pressure in the left ventricle (dP/dt max), measured at a constant heart rate and at a constant mean aortic pressure, was used as an index of inotropic changes in the heart (Furnival, Linden & Snow, 1970). In twenty-five dogs the resting dP/dt max measured $3{,}795 \pm 205$ mmHg/sec (mean; S.E.M.) and the resting heart rate 129 ± 3 beats/min (mean; S.E.M.). In twelve dogs which had received two injections of reserpine (0·5 mg/kg) subcutaneously 24 h apart, the resting dP/dt max measured $2{,}921 \pm 192$ mmHg/sec (mean; S.E.M.) and the resting heart rate 126 ± 4 beats/min (mean; S.E.M.). These results suggest that circulating catecholamines have no significant effect on heart rate but do affect dP/dt max. This suggestion was corroborated in further experiments; propranolol in doses up to 0·05 mg/kg was given to seven dogs which had not been pre-treated with reserpine and dP/dt max was reduced from $3{,}250 \pm 377$ mmHg/sec (mean; S.E.M.) to $2{,}425 \pm 309$ mmHg (mean; S.E.M.) but there was no significant change in heart rate.

In four dogs which had received reserpine the relationship between free heart rate and dP/dt max induced by isoprenaline in the presence of propranolol (0·1–0·5 mg/kg) was the same as in its absence. Thus propranolol produced no change in this relationship. This result is different from the results previously described by Harry, Kappagoda, Linden & Snow (1971) observed in denervated dogs which had not received reserpine; in these dogs the relationship was changed such that for a given heart rate induced by isoprenaline dP/dt max measured less in the presence of propranolol than in its absence.