stimulation (0.5-5 Hz) and the frequency-output slope reduced to near zero. The reverse occurred when the calcium concentration was reduced to 1.27 mM, there was a proportionately greater reduction of output at the lower frequencies of stimulation and the frequency-output slope increased threefold. In the mouse vas deferens raising the calcium concentration from 2.54 to 5.08 mM preferentially increased output at low frequencies, thus causing the output/pulse of noradrenaline to decrease with frequency. Conversely decreasing the calcium concentration to 1.27 mM reduced the output at low frequencies thus causing the output of noradrenaline to increase with frequency. Therefore, modification of the external calcium concentration altered the frequency-output relationship at various sites such that the noradrenaline output/pulse increased, decreased or remained constant with stimulus frequency.

In the rabbit portal vein and vas deferens, tissues in which the output/pulse increased as the frequency was increased (calcium concentration 2.54 mM), angiotensin (0.2-1 $\mu$M) increased the noradrenaline output at low frequencies (0.5-5 Hz), with little or no effect at higher frequencies. Thus in the presence of angiotensin the output/pulse remained constant between 0.5 and 16 Hz. In the mouse vas deferens, angiotensin (1 $\mu$M) did not alter the output/pulse at low or high frequencies. Morphine (1 $\mu$M) depressed the output at low frequencies in the mouse vas deferens so that in the presence of morphine the output/pulse increased with stimulus frequency. In the rabbit portal vein and vas deferens, morphine had no effect.

The possibility therefore arises that differences in the patterns of noradrenaline output may reflect different mechanisms involved in calcium utilization by the release process. Modulation of these mechanisms by drugs can lead to frequency-dependent effects on noradrenaline output.

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## Acetylcholine synthesis from [$^{14}$C]-choline in isolated segments of guinea-pig ileum

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The study of transmitter mechanisms in adrenergic nerves has been greatly facilitated by the use of radio-labelled noradrenaline and its precursors. Few comparable studies have been carried out with radio-labelled acetylcholine or choline. Uptake and release have been investigated with brain synaptosomes (Clouet & Williams, 1974), phrenic nerve-diaphragm preparations (Chang & Lee, 1970; Potter, 1970), autonomic ganglia (Friesen, Kemp & Woodbury, 1965), and heart (Wallach, Goldberg & Shideman, 1967; Buterbaugh & Spratt, 1968). Only one study has been carried out with intestine (Mattila & Idänpään-Heikkilä, 1968).

Longitudinal muscle–Auerbach's plexus preparations were dissected from guinea-pig terminal ileum, as described by Paton & Aboo Zar (1968), and mounted in an organ bath (8 ml) in Krebs-Henseleit solution containing physostigmine (0.05 $\mu$g/ml). The strips were incubated for 75 to 300 min in [$^{14}$C]-choline at a radioactive concentration of 0.125 $\mu$Ci/ml corresponding to choline concentrations of 4.2 to 7.0 $\mu$M; these concentrations of choline did not affect the tone of the muscle. The incorporation of the $^{14}$C-label into acetylcholine in the tissue was determined as described by Potter & Murphy (1967).

Strips which were stimulated at 0.1 Hz during the period of incubation showed increased incorporation of radioactivity attributable to acetylcholine amounting to 171% of that in unstimulated strips ($P < 0.02$, $n = 6$) for a 75 min period of incubation. However, there was a significant decrease to 66% and 70% of control ($P < 0.001$ for each) for periods of incubation of 150 min and 225 min, respectively. These findings suggest that the rate of incorporation of [$^{14}$C]-choline into acetylcholine is increased by field stimulation of cholinergic neurones, but if the incubation time is prolonged the increased turnover of label caused by stimulation leads to loss of choline by metabolic degradation or by incorporation into other molecules such as phospholipids.

Tetrodotoxin (0.2 μg/ml) had no significant effect ($P > 0.2$, $n = 3$) on the formation of [$^{14}$C]-acetylcholine in stimulated preparations, though it abolished the twitch responses to stimulation. However, in non-stimulated preparations the formation of [$^{14}$C]-acetylcholine during the 150 and 225 min periods of incubation was significantly decreased ($P < 0.001$, $n = 6$) by tetrodotoxin.

Hyoscine (0.02 μg/ml) abolished responses to stimulation but had no effect on the incorporation of [$^{14}$C]-choline into acetylcholine in stimulated preparations.

Noradrenaline (1 μg/ml) caused an increase in formation of [$^{14}$C]-acetylcholine which was significant ($P < 0.05$, $n = 3$) in non-stimulated but not in stimulated preparations with a 75 min incubation period. Contractile responses to stimulation were only transiently depressed.

The presence of hemicholinium (50 μg/ml) during the period of incubation with [$^{14}$C]-choline decreased the incorporation of label into acetylcholine to about 12% of control in both stimulated and non-stimulated preparations during the incubation periods studied. This finding is compatible with the view that hemicholinium blocks choline uptake. However, contractile responses to stimulation were not affected by hemicholinium, so it appears that preformed stores of acetylcholine were not exhausted with the regimen of stimulation used.

When strips were washed repeatedly after incubation, the level of radioactivity in the bath fluid fell to a fairly constant level after 20 min. Then, stimulation at 0.1 Hz for 3 min did not lead to an increase in radioactivity in the fluid unless hemicholinium (10 μg/ml) was present.

The findings suggest that [$^{14}$C]-choline was incorporated into acetylcholine, at least part of which was intraneuronal, but the release of the radio-labelled transmitter could only be detected if reuptake of choline (or acetylcholine) was blocked.

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## Definition of the antagonistic action of burimamide and metiamide on the positive inotropic effect of histamine in isolated heart preparations


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Burimamide and metiamide have been defined as histamine $H_2$-receptors antagonists since they were found capable of blocking some mepyramine-insensitive histamine responses, such as the positive chronotropic effect on guinea-pig atria, and the stimulation of gastric acid secretion in the rat (Black, Duncan, Durant, Ganellin & Parsons, 1972; Black, Duncan, Emmet, Ganellin, Hesselbo, Parsons & Wyllie, 1973).

Since it is known that histamine increases both frequency and force of contraction (Mannaioni, 1972) experiments have been carried out to study whether burimamide and metiamide antagonizes the positive inotropic actions of histamine. Moreover the inotropic action of histamine was compared with that of noradrenaline.

Isometric contraction curves and their