Methacholine, a cholinergic agent, stimulates catecholamine release in man

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Abstract. To study the effect of cholinergic stimulation on catecholamine release, methacholine, a choline ester, was injected im into normal subjects. Significant increases in plasma norepinephrine and plasma epinephrine concentrations were observed in response to methacholine administration with significant haemodynamic changes, but there was no correlation between the rise in plasma catecholamines and the haemodynamic changes. These results suggest that the increases in plasma catecholamines induced by methacholine did not result from reflex responses to haemodynamic changes and thus could be a direct effect of methacholine on sympathetic nerves and the adrenal medulla.

Although it is well known that pre- to post-ganglionic neurotransmission is cholinergic, and cholinergic stimulation has been reported to stimulate catecholamine release from the adrenal medulla in animals (Lewis 1975), there have been few reports of cholinergic stimulation of catecholamine release in human subjects.

Dopamine-β-hydroxylase (DBH) is discharged from sympathoadrenal cells into the circulation together with catecholamines by exocytosis and many recent reports have explored the clinical value of plasma DBH activity in the assessment of human sympathetic nervous system function (Weinshilboim & Axelrod 1971; Ziegler et al. 1977).

Previously we confirmed that plasma DBH activity increases in response to an im injection of methacholine (Okada et al. 1977), a choline ester which has been used as a clinical test of human autonomic nervous system function (Funkenstein et al. 1951; Miura 1967). However some results cast doubt on the suitability of plasma DBH activity measurements for evaluating human sympathetic activity (Lake et al. 1977).

This study was undertaken, however, to investigate the effect of methacholine on plasma norpinephrine and epinephrine concentrations in normal subjects, using high speed ion-exchange column chromatography and an automated trihydroxyindole (THI) method.

Materials and Methods

Five men, 30–42 years old, were selected for testing. All subjects were examined clinically and found to be normotensive with no evidence of clinical disease. They denied the use of drugs. Consent was obtained from each subject after full explanation of the purpose, nature and risks of all procedures used, and the Committee of Department of Internal Medicine, Fukushima Medical College, in which the study was performed, approved the protocol.

Studies were performed in a quiet, darkened room with subjects in a overnight fasted state, and resting in the supine position. Venous blood samples were drawn through an iv sampling needle inserted into an antecubital vein 30 min before sampling. Blood samples were drawn at -10, 0, 1, 3, 5, 7, 10, 15 and 20 min in relation...
to an im injection of methacholine (acetyl-β-methylcholine chloride, Daiichi Kagaku Yakuin Co., Tokyo), 10 mg. Systolic blood pressure and pulse rate were recorded automatically at all sampling points by an ultrasonic blood pressure monitor (Arteriosonde® 1225, Roche, USA).

Plasma catecholamine were determined using high speed ion-exchange column chromatography and an automated fluorimetric THI method which is of higher specificity and of greater accuracy than the aluminium oxide THI procedure which was frequently used in the past.

Alumina (Woelm, neural activity, grade I), 100 mM EDTA-2Na, 0.4 M HClO₄, 7% NH₄OH, 0.2 M CH₃COOH, 0.3 M CH₃COOH, 200 mM KH₂PO₄, 200 mM K₂HPO₄, 7.6 mM K₃Fe(CN)₆, 14.2 mM ascorbic acid and 5 M NaOH were prepared as reagents. All chemicals used were of analytical grade. Hitachi 635A liquid chromatograph, Hitachi 3011-C resin (acidic, cation ion exchange resin) pack in 2.6 mm × 250 mm column and an ultraviolet detector (Hitachi 650-10LC fluorescence spectrophotometer) were used. The procedure was as follows. Two hundred μl of 100 mM EDTA-2Na and 2 ml of 0.4 M HClO₄ were added to 2 ml of plasma. After centrifugation at 10 000 g for 10 min, the supernate was decanted to another tube, adjusted to pH 8.6 with 7% NH₄OH, and 500 mg of alumina was added. The mixture was shaken gently for 5 min and the precipitated alumina was washed with 10 ml of distilled water. Then catecholamines were eluted with 5 ml of 0.3 M CH₃COOH and were lyophilized. After lyophilization, catecholamines were eluted again with 10 μl of 0.2 M CH₃COOH and 50

![Graph showing effect of methacholine injection on plasma catecholamines concentration, systolic blood pressure, and pulse rate.](image-url)

**Fig. 1.**

Effect of an im injection of methacholine at a dose of 10 mg on plasma norepinephrine concentration, epinephrine concentration, systolic blood pressure and pulse rate in 5 normal subjects. Each point with a vertical line attached is the mean value ± 1 se. P-values are shown vs zero time value (*P < 0.05, **P < 0.02, ***P < 0.01).


μl of aliquot was injected for high speed liquid chromatography. The catecholamines were continuously eluted from the column with 200 mM KH2PO4 at 0.6 ml/min of flow rate and the reagents necessary for formation of the fluorescent trihydroxindole derivatives, namely 100 mM KH2PO4, 200 mM K2HPO4 and 7.6 mM K3Fe(CN)6 followed by 14.2 mM ascorbic acid and 5 N NaOH, were added in appropriate amounts and in the correct sequence. Fluorescence wavelengths were adjusted to 415 nm of excitation and 510 nm of emission. Results showed the high specificity for catecholamine of this assay system. Linearity between quantity and the height of each peak was observed with norepinephrine and epinephrine in the range from 10 pg to 1000 pg/ml. A plasma sample was measured repeatedly on different days and the reproducibility was calculated. The standard deviations for norepinephrine and epinephrine were 8.2% and 10.1%, respectively.

Statistical significance was determined by means of the paired Student's t-test.

Results

Mean (± SE) plasma norepinephrine and epinephrine concentrations, systolic blood pressure and pulse rate before and after the injection of methacholine into five normal subjects are shown in Fig. 1.

After the injection of methacholine all subjects had symptoms referable to cholinergic stimulation such as facial flushing, salivation, sweating and palpitation, but these symptoms did not cause the subjects undue distress.

Plasma norepinephrine rose from an initial value of 65.8 ± 6.1 to a maximum of 125.0 ± 13.6 pg/ml 3 min after methacholine injection and was significantly higher than the zero time value from 3 min to 5 min (P < 0.01). Plasma epinephrine rose significantly from 32.8 ± 8.0 to a maximum of 62.8 ± 8.7 pg/ml 3 min after methacholine injection (P < 0.02). Mean systolic blood pressure decreased significantly from 105.0 ± 2.0 to 101.1 ± 3.0 mmHg 1 min after methacholine injection (P < 0.05) and increased significantly to 109.2 ± 3.4 at 7 min (P < 0.05), and 111.8 ± 3.0 mmHg (P < 0.01) 10 min after methacholine injection. Mean pulse rate increased from 61.6 ± 2.0 of zero time value to 75.2 ± 5.3, and 76.0 ± 4.1/min at 1 min and 3 min after methacholine injection, respectively (P < 0.05).

Increments in plasma norepinephrine and decrements in systolic blood pressure after methacholine injection in individual subjects were not correlated (r = −0.32, NS). Increments in plasma epinephrine and apparent decrements in systolic blood pressure after methacholine injection in individual subjects did not correlate either (r = −0.25, NS) (Fig. 2).

Discussion

Leveston et al. (1979) reported that cholinergic stimulation with edrophonium stimulates catecholamine release in man. They observed that significant though briefly sustained increments in plasma
catecholamines were not attributable to reflex responses to haemodynamic changes and that similar increments in plasma norepinephrine occurred in adrenalectomized patients. It was concluded that in man cholinergic activation with edrophonium results in direct stimulation both of sympathetic postganglionic neurons, with augmented norepinephrine release, and of the adrenal medulla, with augmented epinephrine release (Leveston et al. 1979).

Unlike edrophonium, an acetylcholinesterase inhibitor widely used in diagnostic testing for myasthenia gravis, methacholine is a choline ester with a different mechanism of cholinergic action comparable to acetylcholine, and has been used clinically to assess human sympathetic nervous system function (Funkenstein et al. 1951; Miura 1967). Methacholine has a longer action than acetylcholine and has a good safety record.

Funkenstein et al. (1951) were the first to use methacholine to study human sympathetic nervous system function. Gellhorn (1957) investigated the physiological nature of the findings of Funkenstein et al. (1951) and postulated that a methacholine-induced fall in blood pressure elicits a reflex sympathetic discharge from the hypothalamus, so that the methacholine test probably indicates the degree of hypothalamic sympathetic excitability in human subjects. Since then blood pressure determination has been only one among several physiological variables used as measures of sympathetic nervous system activity in the methacholine test. This has largely been due to the lack of specific and practical means for the determination of catecholamines.

DBH released by exocytosis from sympathetic adrenal cells along with catecholamines has been considered the primary source of circulating enzyme, and thus plasma DBH activity should reflect changes in sympathoadrenal function (Weinshilboum & Axelrod 1971). We reported that an im injection of metacholine caused a significant increase in plasma DBH activity, and suggested that the determination of the plasma DBH activity response to cholinergic activation with methacholine would be a good measure of human sympathetic nervous system function (Okada et al. 1977). However some reports question the suitability of plasma DBH activity measurements for assessing human sympathetic nervous system function (Horowitz et al. 1973; Kopin et al. 1976), and indeed a lack of correlation between plasma DBH activity and norepinephrine level has been reported (Lake et al. 1977).

In this study we used high speed liquid chromatography and the automated THI method for plasma catecholamines, and found that methacholine induced significant increases in plasma norepinephrine and epinephrine with haemodynamic changes. The decrease in systolic blood pressure and the increase in pulse rate slightly preceded significant increases in plasma catecholamines. Though it is generally accepted that decrements in pulse rate, blood pressure and pulse pressure activate catecholamine release (Kirshheim 1976), the increments in plasma catecholamines were not correlated with apparent decrements in systolic blood pressure in our study. Therefore the increments in plasma catecholamines induced by methacholine were not attributable to reflex responses to haemodynamic changes and could rather be a direct effect of methacholine on sympathetic nerves and the adrenal medulla.

This is the first report that methacholine administration increases in plasma norepinephrine and epinephrine concentrations in man. Measurements of plasma catecholamine responses to cholinergic stimulation with methacholine may present a new approach to the study of human sympathetic nervous system function, including a re-evaluation of methacholine test in spite of the different mechanism of cholinergic action from edrophonium.

References


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