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Detection of Endogenous Acetylcholine Release from the Rat Basal Forebrain Slices

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〔第60回 日本薬理学会総会 (昭和62年 4月1日, 千葉市) で発表〕

We have developed a specific highly sensitive radioimmunoassay for acetylcholine (ACh). In the present study, an attempt was made to determine endogenous ACh released from the rat basal forebrain slices. The brain slices (40—60 mg protein) were placed in the perfusion chamber (0.3 ml) and perfused with artificial cerebrospinal fluid (37°C, 0.4 ml/min). Fractions were collected every 3 min and determined for ACh contents. In the normal condition (without cholinesterase (ChE) inhibitor), no detectable amount of ACh was present in the superfusates. Thus, a medium containing methanesulfonyl-fluoride (10 μ M) was perfused to inhibit ChE. Under these conditions, spontaneous release of ACh was detected (0.56 ± 0.04 pg/mg protein/min). Atropine-sulfate (up to 10 μ M) and pirenzepine-hydrochloride (1 μ M) evoked an increase in ACh release. Thus, M_1 -muscarinic autoreceptor seems to be activated by spontaneously released ACh in the presence of ChE inhibitor.

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Determination of Plasma Acetylcholine Concentrations in Rabbits and Humans

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〔第60回 日本薬理学会総会 (昭和62年 4月1日, 千葉市) で発表〕

It is generally considered that acetylcholine (ACh) is rapidly hydrolyzed by acetylcholinesterase (AChE), and no detectable amount is present in the blood. By using a specific and sensitive radioimmunoassay (RIA) for ACh, the present study was conducted to confirm whether there is any measurable amount of ACh in plasma. Venous blood sample was collected into a cooled vacutainer containing EDTA, paraoxon and acetic

acid. Plasma was separated by centrifugation and was applied on to a Centricon-10®. The filtrate was obtained by centrifugation at 5000 × g. A 100 μl portion of the filtrate was used for RIA. The filtrate subjected to alkaline hydrolysis served as blank. Under these bleeding conditions, AChE activity in the blood was confirmed to be completely inhibited. The recovery ratio of ACh added to plasma was 103.6±5.0%. Plasma concentrations of ACh were 639±60 in rabbits (n=6), 526±68 in men (n=6) and 542±115 pg/ml in women (n=8). These data demonstrate that small amount of ACh is present in the blood of rabbits and humans. The origin of ACh in the blood is under investigation.

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Enhancement of Acetylcholine Release and Contraction Response by Pirenzepine and Atropine in the Longitudinal Muscle Strips of Guinea-pig Ileum

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〔第60回 日本薬理学会総会 (昭和62年4月1日, 千葉市) で発表〕

We have recently shown that acetylcholine (ACh) released from the longitudinal muscle strips of guinea-pig ileum can be directly measured by a radioimmunoassay (RIA). The strips were suspended in an organ bath and perfused with normal or drug containing Krebs solution (0.4 ml/min). The strips were pretreated with methanesulfonyl fluoride, an irreversible cholinesterase (ChE) inhibitor. Electrical stimulation produced a contraction response and an increase in ACh release. Perfusion with pirenzepine (0.1—10 μM) and atropine (1—100 nM) increased ACh release from the strips upon electrical stimulation. The contraction response was enhanced by perfusion with lower concentrations of both pirenzepine (0.1 and 1 μM) and atropine (1 and 10 nM), while the enhancement of the contraction response was abolished at higher concentrations of these drugs. The data indicate that pirenzepine and atropine increase ACh release through the action on M₁-muscarinic autoreceptors under the present experimental conditions. M₁-muscarinic receptor in the myenteric neurons appears to be involved in the regulation of ACh release.

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