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## Radioimmunoassay for Acetylcholine in the Brain and the Suprachiasmatic Nucleus of the Rat.

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[第59回 日本薬理学会総会 (昭和61年 4月2日, 新潟) で発表]

Several procedures have been successfully applied to the determination of acetylcholine (ACh) in the tissue. This paper wants to present procedures for the development of radioimmunoassay (RIA) for ACh and its application to the determination of ACh in the various brain regions of the rat.

*Development of RIA for ACh.* Antiserum against ACh was raised in rabbit immunized with the conjugates of bovine serum albumin with choline hemiglutarate. The antiserum was specific for ACh and butyrylcholine. Its cross-reactivities with choline and choline phosphates, such as phosphatidylcholine and phosphorylcholine were negligibly small (less than 0.012%). Using the antiserum (final dilution 1 : 15,000) and [ $^3\text{H}$ ]ACh (77 Ci/mmol, Amersham), the assay in 0.15 M Tris-HCl buffer can reliably determine as little as 10 pg/tube of ACh and the standard curve is linear up to 1000 pg/tube.

*ACh contents in the various brain regions of the rat.* Rats were killed by irradiation with a microwave beam onto the head. The brain was removed and dissected into 6 regions. The tissues were homogenized in 59 volumes of 0.4 M perchloric acid. The homogenate was centrifuged to separate the supernatant. To prepare blank sample, 300  $\mu\text{l}$  portion of the supernatant was subjected to alkaline hydrolysis. The other 300  $\mu\text{l}$  of the supernatant was diluted with 900  $\mu\text{l}$  of 0.17 M NaCl solution to make the salt concentration comparable with that of the blank sample. To the assay tubes containing the diluted antiserum, Tris-HCl buffer and [ $^3\text{H}$ ]ACh was added 10  $\mu\text{l}$  portion of the sample, the blank or standard ACh solution. ACh content of the supernatant was calculated by subtracting the blank value. Usually blank sample did not affect [ $^3\text{H}$ ]ACh binding to the antibody. ACh contents in the anterior hypothalamus and the medulla oblongata of the adult SHR were found to be significantly lower than those of the WKY.

*ACh content in suprachiasmatic nucleus (SCN).* After the microwave treatment, the brain was removed and sliced at about 800  $\mu\text{m}$  of thickness. The SCN was punched out with a glass tubing having an inside diameter of 700  $\mu\text{m}$ . The tissue was homogenized in 300  $\mu\text{l}$  of 0.4 M perchloric acid. The supernatant was treated as described above. ACh content in the SCN was found to be influenced by light.

*Summary.* Advantages of RIA for ACh are specificity, sensitivity and simplicity of operation. With this RIA, it is possible to determine 10 pg of ACh in the supernatant of

the brain homogenate without the need for extraction.

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### Effect of IPS-339, a $\beta_2$ -Antagonist, on Physostigmine-induced Hypertension in the Rat.

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[第59回 日本薬理学会総会 (昭和61年4月4日, 新潟) で発表]

Physostigmine (PHY) produces a hypertension by activation of sympathetic nervous system as a result of central stimulation of muscarinic receptors. Involvement of  $\beta_2$ -adrenoceptors in PHY-induced hypertension was examined in conscious, unrestrained rats. Two days before the experiment, male Wistar rats were instrumented with a chronic aortic catheter for direct recordings of blood pressure and heart rate. PHY (100  $\mu$ g/kg, ia) was administered twice; firstly, 30 min after IPS-339, other  $\beta$ -antagonists or saline injection to examine drug effects. IPS-339 at the doses of 250 and 500  $\mu$ g/kg did not affect hypertensive response to PHY. PHY-induced hypertension was significantly attenuated by bunitrolol, metoprolol, and propranolol at the dose of 250  $\mu$ g/kg while it was augmented or unchanged by these drugs at the dose of 500  $\mu$ g/kg. These results indicate that  $\beta_2$ -adrenoceptors are not involved in the PHY-induced hypertension. Since the low dose of bunitrolol, metoprolol and propranolol attenuated the hypertensive response to PHY,  $\beta_2$ -adrenoceptors appear to play some role in the response.

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### モルモット腸管からの遊離されるアセチルコリンの定量

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[第74回 日本薬理学会関東部会 (昭和61年6月7日, 神奈川県) で発表]

モルモット回腸縦走筋標本を電気刺激すると副交感神経末端よりアセチルコリン (ACh) が遊離され, 収縮反応が観察される。これまで ACh 遊離量は別の回腸標本またはヒルの背筋を用いるバイオアッセイにより定量されてきた。しかしながら, 平滑筋の収縮反応に影響をおよぼす薬物などの ACh 遊離に対する作用の検討は, これらのバイオアッセイでは不可能であった。そこで我々はラジオイムノアッセイ (RIA) を用いてモルモット回腸縦走筋標本から遊離される ACh の直接定量を試みた。