Stimulation of α- and β-Receptors in the Isolated Rat Jejunum

It has been shown that stimulation of either α- or β-receptors produces relaxation of the intestine in the canine ileum, mouse ileum, and rat duodenum[1-4]. On the other hand, it has been found that adrenaline contracts the intestine in the terminal portion of the guinea-pig ileum[5,6]. CHRUSCIEL et al.[7] reported a triphasic intestinal reaction to adrenaline in the rat, which consisted of an initial relaxation and a secondary increase in the tone followed by a third relaxation. The present paper describes how α-receptor stimulation produces both relaxation and contraction, and β-receptor stimulation produces relaxation of the rat jejunum.

Materials and methods. Rats of either sex weighing 160–260 g were killed by bleeding after a blow on the head. Segments of the jejunum about 2 cm in length were suspended in the bath containing 20 ml of the Gaddum solution. The organ bath was bubbled with 100% oxygen, and was maintained at 27 °C. Adrenaline, isoproterenol and phenylephrine were used as agonists, and propranolol and dibenamine were used as antagonists.

Results. Adrenaline usually produced a triphasic response after the treatment of the jejunum with propranolol. Figure 1 shows a schematic illustration of the response, in which the maximal relaxation is expressed by h_1 and maximal contraction is expressed by h_2. As the responses to isoproterenol and phenylephrine were similar to the response to adrenaline, h_1 and h_2 were measured not only in the case of adrenaline but also in the cases of isoproterenol and phenylephrine. The effects of propranolol and dibenamine on h_1 and h_2 are summarized in the Table.

Adrenaline in the concentration of 5×10^{-8} g/ml caused relaxation of the jejunum. During the relaxation, it was occasionally observed that the tone of the jejunum increased slightly, then relaxed again. When propranolol in the concentration of 2.5×10^{-7} g/ml was added to the bath about 5 min before the addition of adrenaline, the degree of relaxation to be induced by adrenaline was inhibited and that of contraction was increased (or the phase of contraction appeared in the experiments where adrenaline-induced contraction was not observed in the absence of propranolol) as seen in rat A of Figure 2. When dibenamine in the concentration of 2.5×10^{-7} g/ml was added to the bath about 30 min before adrenaline, the degree of relaxation seemed to increase, and the phase of slight contraction disappeared (Figure 2, rat B).

Isoproterenol in the concentration of 5×10^{-9} g/ml caused relaxation of the rat jejunum without producing contraction. By the previous addition of 2.5×10^{-7} g/ml of propranolol, the effect of isoproterenol on the jejunum was completely blocked. A slight contraction, however, was occasionally observed by the addition of isoproterenol in the presence of propranolol. Dibenamine in the concentration of 2.5×10^{-7} g/ml had a tendency to increase the degree of relaxation induced by isoproterenol.

Phenylephrine in the concentration of 5×10^{-7} g/ml caused relaxation followed by contraction in most cases of the experiments. This reaction resembles that induced by adrenaline in the presence of propranolol. In some preparations, only the phase of contraction was observed by the addition of phenylephrine. Propranolol in the concentration of 2.5×10^{-7} g/ml did not affect the degree of relaxation induced by phenylephrine, but it inhibited the

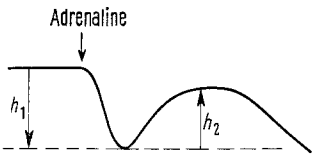

Fig. 1. Schematic illustration of the effect of adrenaline on the isolated rat jejunum pretreated with propranolol. The maximal degree of relaxation and contraction are shown by h_1 and h_2, respectively.

[1] R. P. AHLQUIST and B. LEVY, J. Pharmac. exp. Ther. *127*, 146 (1959).
[2] B. LEVY, J. Pharmac. exp. Ther. *127*, 150 (1959).
[3] K. TAKAGI, E. OSADA, I. TAKAYANAGI and F. TAGA, Archs int. Pharmacodyn. Thér. *168*, 212 (1967).
[4] B. LEVY, Archs int. Pharmacodyn. Thér. *170*, 418 (1967).
[5] A. KAWAI, J. Osaka med. Coll. *22*, 263 (1964).
[6] A. KAWAI, J. Osaka med. Coll. *22*, 271 (1964).
[7] T. L. CHRUSCIEL and S. M. POJDA, Experientia *24*, 1227 (1968).

Effects of antagonists on h_1 and h_2 (see Figure 1) produced by agonists

Agonists	Antagonists							
	Propranolol (2.5×10^{-7} g/ml)				Dibenamine (2.5×10^{-7} g/ml)			
	Control		After propranolol		Control		After dibenamine	
	h_1 (mm)	h_2 (mm)	h_1 (mm)	h_2 (mm)	h_1 (mm)	h_2 (mm)	h_1 (mm)	h_2 (mm)
Adrenaline (5×10^{-8} g/ml)	—20.96 ± 2.57 (8)	+ 0.80 ± 0.80 (8)	—9.02 ±1.09 (8)	+10.53 ± 2.94 (8)	—27.67 ± 8.92 (5)	0 (5)	—34.81 ± 4.54 (5)	0 (5)
Isoproterenol (5×10^{-9} g/ml)	—14.81 ± 4.07 (4)	0 (4)	0 (4)	+ 3.90 ± 3.09 (4)	—20.25 ± 2.44 (4)	0 (4)	—24.54 ± 2.96 (4)	0 (4)
Phenylephrine (5×10^{-7} g/ml)	— 4.09 ± 1.45 (8)	+23.40 ± 4.22 (8)	—4.84 ±1.22 (8)	+16.38 ± 3.01 (8)	— 8.03 ± 4.59 (8)	+17.86 ± 4.07 (8)	— 0.38 ± 0.38 (8)	0 (8)

The degrees of h_1 and h_2 are expressed by mm change (with standard error) on the smoked paper. Number of preparations tested is shown in parentheses.

degree of contraction. This blocking effect of propranolol on the phenylephrine-induced contraction seemed to be non-specific, because the same concentration of propranolol also inhibited the contraction induced by serotonin. When dibenamine in the concentration of 2.5×10^{-7} g/ml was added to the bath 30 min before phenylephrine, both phases of relaxation (if present) and contraction were completely abolished.

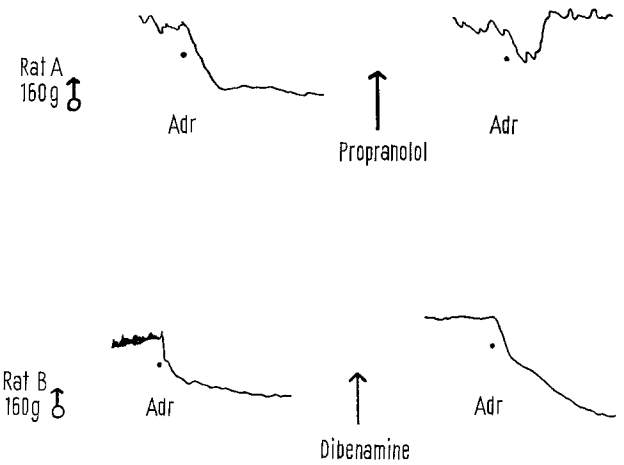

Fig. 2. Change in response to 5×10^{-8} g/ml of adrenaline (Adr) in the presence of 2.5×10^{-7} g/ml of propranolol (Rat A), and that in the presence of 2.5×10^{-7} g/ml of dibenamine (Rat B).

The foregoing results suggest that stimulation of α-receptors produces an initial relaxation followed by a secondary contraction in the rat jejunum, because this typical response can be seen by the addition of adrenaline in the presence of propranolol. Phenylephrine also produced similar response. The fact that this type of reaction to adrenaline, or to phenylephrine is almost completely abolished by the pretreatment of the jejunum with dibenamine, indicates that the response mentioned above is produced by stimulation of α-receptors. It is apparent that stimulation of β-receptors produces relaxation of the jejunum, because in our experiment isoproterenol produced relaxation, which was not inhibited by the pretreatment with dibenamine but was completely abolished by propranolol. So, the relaxation of the rat jejunum could be produced by stimulation of either α- or β-receptors. But the contraction could be produced by stimulation of α-receptors.

Zusammenfassung. Es wird gezeigt, dass die Stimulation der α-Rezeptoren am isolierten Rattenjejunum Erschlaffung und Kontraktion verursacht, während die Stimulation der β-Rezeptoren nur Erschlaffung bewirkt.

A. MINAMIDATE

Department of Pharmacology, Fukushima Medical College, Fukushima (Japan), 10 January 1970.

Autoradiographic Localization of 5-Hydroxytryptamine in Monkey Pineal Gland

The pineal gland of man and other mammals contains a high concentration of 5-hydroxytryptamine (5-HT) and the highest levels recorded have been in the rat and monkey[1,2]. It appears[3] that the principal intrapineal pathway for the metabolism of 5-HT depends on the activity of the enzyme monoamine oxidase (MAO) which is present in large amounts in pineal gland tissue[4]. The present report deals with a morphological study of the monkey pineal gland employing an autoradiographic technique. The investigation is concerned with first, demonstrating in vivo uptake of tritium-labelled 5-hydroxytryptophan (^{3}H-5-HTP) and its localization within the gland, secondly, the conversion of ^{3}H-5-HTP to ^{3}H-5-HT and thirdly, detecting alterations in intrapineal levels of isotope following interference with MAO activity. Because the level of 5-HT in monkey pineal gland fluctuates over a 24 h period, being at its highest concentration during the daily light period[1], an appropriate time in the morning was chosen for the administration of the isotope.

Three healthy adult male cynomolgus monkeys, *Macaca irus*, housed at the Commonwealth Serum Laboratories, Melbourne, weighing 4000–5000 g each, were used. 2 animals were given 5 mCi/kg ^{3}H-5-HTP[5], specific activity 3.3 Ci/m*M*, at 11.00 h. Previously 1 of the animals received at 10.00 h 20 mg/kg tranylcypromine sulphate, a rapidly acting MAO inhibitor[6]. Both substances were administered by i.p. injection. Tissues from the third animal, which was not injected, were used as controls to exclude possible chemical artefacts in the nuclear emulsion of the autoradiographs. 2 h after the administration of ^{3}H-5-HTP the monkeys were exsanguinated under deep i.v. barbiturate anaesthesia and the pineal glands removed. After fixation in 10% (v/v) phosphate-buffered formol-saline, pH 7.0, the glands were dehydrated in ethanol, embedded in paraffin wax, cut serially at 4 μ thickness and mounted on acid-cleaned glass microscope slides. Sections were dewaxed, dipped in liquid nuclear emulsion (Ilford K5) diluted 1:4 with glass-distilled water and exposed for 10 weeks at 4 °C. The slides were developed for 4 min in Neutol-S (Agfa) diluted with water (1:7), fixed in 'Amfix' solution (May and Baker) for 3 min and then washed for 20 min in filtered tap water. The sections were stained with nuclear fast red and tartrazin O (Chroma) and mounted in polystyrene.

Precise localization of radioactivity was demonstrated over the pineal gland (Figure A). Very few silver grains were present over pinealocytes in the autoradiographs prepared from the monkey given ^{3}H-5-HTP alone (Figure B), but in those prepared from the animal given MAO inhibitor, in addition to ^{3}H-5-HTP, dense collections of grains had developed over these cells (Figure C).

[1] W. B. QUAY, Proc. Soc. exp. Biol. and Med. *121*, 946 (1966).
[2] R. J. WURTMAN, J. AXELROD and D. E. KELLY, *The Pineal* (Academic Press, New York 1968), p. 63.
[3] W. B. QUAY, Proc. Soc. exp. Biol. and Med. *115*, 710 (1964).
[4] R. J. WURTMAN, J. AXELROD and L. S. PHILLIPS, Science *142*, 1071 (1963).
[5] Obtained from the Radiochemical Centre, Amersham, Bucks.
[6] Donated by Smith Kline and French Laboratories.