Br. J. Pharmac. (1979), **66**, 405–408

THE ACTIONS OF 5-HYDROXYTRYPTAMINE AND HISTAMINE ON THE ISOLATED ILEUM OF THE TREE SHREW (*Tupaia glis*)

K. SAKAI, Y. SHIRAKI, T. TATSUMI* & K. TSUJI*

Department of Pharmacology and *Laboratory Animal Centre, Research Laboratories, Chugai Pharmaceutical Co., Ltd., Takada, Toshima-ku, Tokyo 171, Japan

1. Contractions to 5-hydroxytryptamine (5-HT) and histamine of longitudinal muscle from the isolated ileum of the tree shrew (*Tupaia*), guinea-pig and rat were investigated by constructing dose-response curves and studying the effects of various antagonists.

2 In the *Tupaia* and rat ileum the contraction to 5-HT was reduced by methysergide but not affected by tetrodotoxin (TTX), morphine, hexamethonium (C_6) or atropine. The response of guinea-pig ileum to 5-HT was not significantly inhibited by methysergide or C_6, but was blocked by TTX, morphine and atropine.

3 Histamine-induced contraction of *Tupaia* and guinea-pig ileum was antagonized by diphenhydramine but not by TTX, morphine, C_6 or atropine. Histamine was almost without effect on the rat ileum.

Introduction

Tree shrews (e.g. *Tupaia glis*) have an anatomical (Le Gros Clark, 1971) and serological (Goodman, 1966; Moore & Goodman, 1968) resemblance to primitive primates. They appear to have several desirable qualities as experimental animals (Schwaier, 1973; 1975), but little is known of their usefulness in applied medical research.

The present study compares and contrasts the effects of 5-hydroxytryptamine (5-HT) and histamine on the ileum of *Tupaia*, guinea-pig and rat.

Methods

Male adult Sprague–Dawley rats, Hartley guinea-pigs and *Tupaia* (bred in the Chugai Farm) were killed by a blow on the head. The ileum was excised and segments about 2 cm long were removed from the mid-ileum. The oral end of each segment was tied to a supporting hook and the other end to a recording lever. Each preparation was suspended under a load of 0.5 g in a 10 ml organ bath containing Krebs solution at 37°C gassed with 95% O_2 and 5% CO_2. Isotonic muscle contractions were recorded on a pen recorder (Custom Demand Recorder, Model CDR-12A, TOA Electronics Ltd.) using a transducer (Medical Electronics Commercial, ME-4012). The composition of the Krebs solution was (mM): NaCl 119, KCl 1.8, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 11.0. The pH of the gassed solution was 7.4.

Preparations were equilibrated for at least 1 h, with washes every 10 min, before exposure to drugs. Cumulative contractile responses (unless otherwise stated) to 5-HT and histamine were calculated as a percentage of the maximal contraction to acetylcholine. Doses were increased by a factor of about 3, and were introduced when the preceding ones had reached a steady value. Concentration–percentage maximal response curves for 5-HT and for histamine were constructed employing five or six doses. The bath fluid was then changed and the antagonist added to the replacement fluid. After 5 min, the dose–response curves were repeated. A different piece of ileum was used for each experiment.

Drugs used were: 5-hydroxytryptamine creatinine sulphate (Sigma), histamine dihydrochloride (Wako Junyaku), acetylcholine chloride (Daiichi Seiyaku), atropine sulphate (Takeda), tetrodotoxin, morphine (both Sankyo), hexamethonium bromide (Yamanouchi), methysergide tartrate (Sandoz) and diphenhydramine hydrochloride (Kowa). All drugs were dissolved in 0.15 M NaCl and usually injected into the bath in a volume of 0.1 ml.

Values in the test are arithmetic means ± s.e. (unless otherwise stated). The statistical significance of the differences between mean values was assessed by Student's *t* test and expressed as *P* values.

0007-1188/79/070405-04 $01.00

© Macmillan Journals Ltd 1979

Results

5-Hydroxytryptamine

5-HT (0.03 to 10 μM) caused reproducible contractions of the ileum of *Tupaia*, guinea-pig and rat. No tachyphylaxis was observed when the bath fluid was changed six times between each cumulative dose-response curve for 5-HT constructed at 10-min intervals. Figure 1 shows the effects of TTX (2 μM), morphine (0.3 μM), C_6 (100 μM), methysergide (0.03 μM) and atropine (0.03 μM) on the dose-response curve to 5-HT. In preparations of guinea-pig ileum, C_6 and methysergide caused no significant ($P > 0.05$) shift in the dose-response curve to 5-HT. The dose of C_6 (100 μM) was sufficient to shift the dose-response curve to dimethylphenylpiperazinium (1 to 100 μM) about 30 times to the right. Morphine shifted the 5-HT curve to the right. Atropine or TTX caused a pronounced depression of responses to higher doses of 5-HT. In preparations of *Tupaia* and rat ileum, methysergide antagonized the contractile response to 5-HT, whereas TTX, morphine, C_6 or atropine caused no significant ($P > 0.05$) change.

Histamine

Histamine (0.1 to 30 μM) contracted *Tupaia* and guinea-pig ileum, but was almost without effect on the rat ileum. The contraction caused by histamine in *Tupaia* and guinea-pig ileum preparations was not affected by TTX (2 μM), morphine (0.3 μM), C_6 (100 μM) or atropine (0.03 μM). On the other hand, diphen-

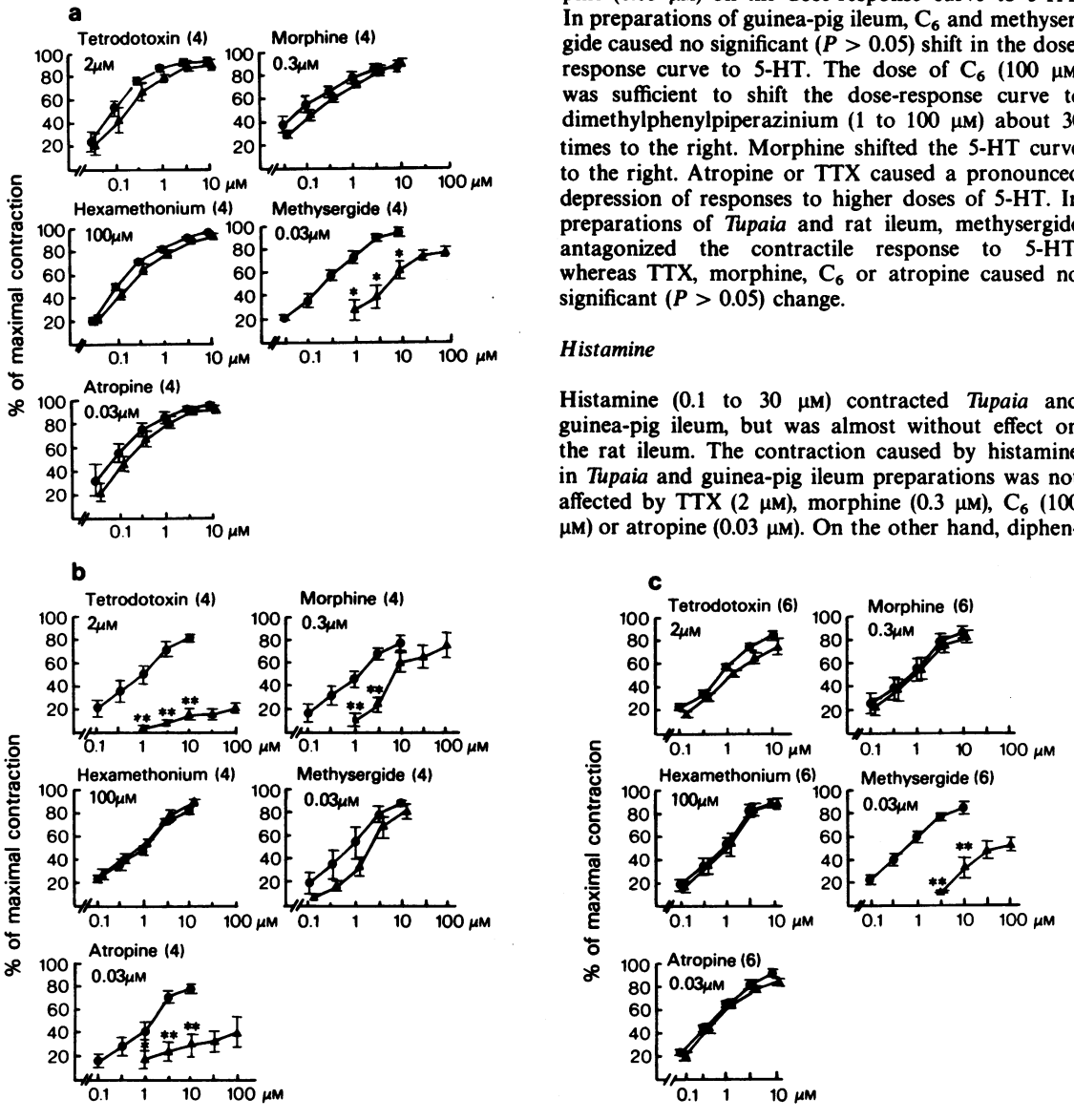

Figure 1 The effects of five antagonists on longitudinal muscle contractions to 5-hydroxytryptamine (5-HT) in *Tupaia* (a), guinea-pig (b) and rat (c) ileum. 5-HT was cumulatively added to the bath fluid, except in methysergide-treated preparations of *Tupaia* and rat ileums. In *Tupaia* and rat ileum, the effect of methysergide was tested with single doses of 5-HT, because the response to 5-HT reached a maximum and fell rapidly in the treated preparations. Responses to agonists are plotted as % maximal contraction to acetylcholine against the $\log_{10}$ molar concentration. Each curve represents mean ± s.e. and number of experiments is shown in parentheses. Circles show responses to agonists and triangles the effect of antagonists. Significant differences are shown as follows: * $P < 0.05$; ** $P < 0.01$.

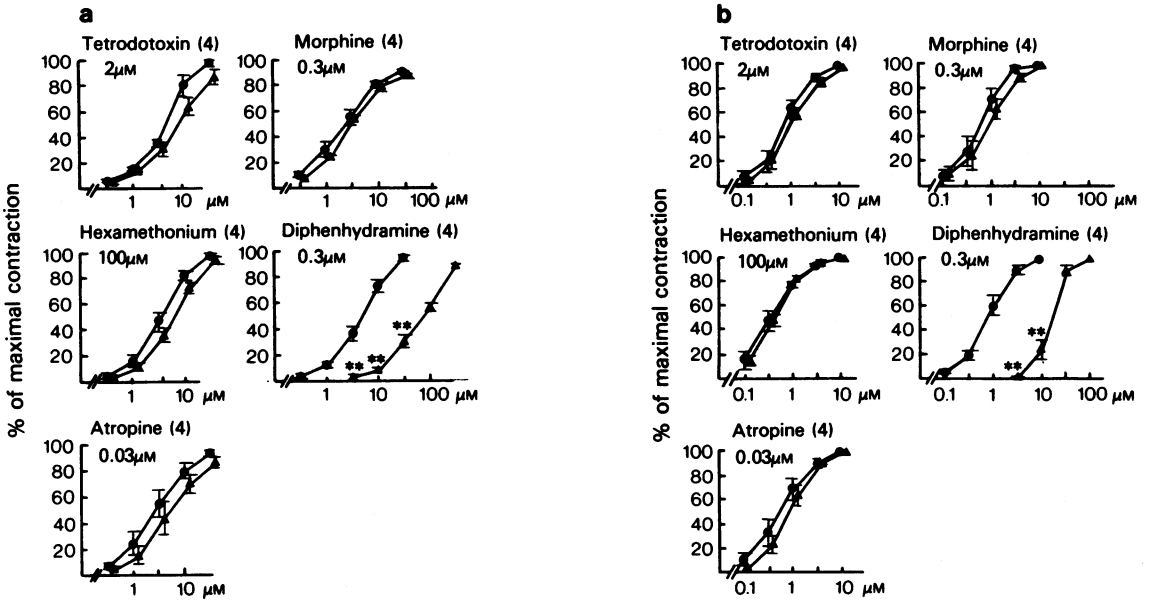

Figure 2 The effects of five antagonists on longitudinal muscle contractions to histamine in *Tupaia* (a) and guinea-pig (b) ileum. Histamine was almost inactive on rat ileum even at the highest concentration (30 μM) used. Other details as in Figure 1.

hydramine (0.3 μM) antagonized the histamine-induced contraction. Summarized data are shown in Figure 2.

Discussion

The contractile response of *Tupaia* isolated ileum to 5-HT was antagonized by methysergide, a substance which blocks smooth muscle 5-HT receptors (Day & Vane, 1963) but was not significantly affected by TTX, morphine or C_6. Thus, 5-HT seems to act on ileal longitudinal muscle fibres but not on nerves.

The site of action of 5-HT in *Tupaia* ileum was similar to that in longitudinal muscle of rat ileum. It differed from that in guinea-pig ileum, where TTX, atropine or morphine inhibited the contraction to 5-HT. This finding points to the involvement of neural elements in induction of the contraction, and supports previous conclusions concerning the site of action of 5-HT (Rocha e Silva, Valle & Picarelli, 1953; Robertson, 1953; Gaddum & Hameed, 1954; Gaddum & Picarelli, 1957; Brownlee & Johnson, 1963; Day & Vane, 1963).

The mode of action of histamine on the ileum was similar in the *Tupaia* and guinea-pig, but differed in the rat which was virtually unaffected. Since the histamine-induced contraction in *Tupaia* or guinea-pig was not modified by TTX, morphine or C_6, it is not mediated through any neural element. Presumably, histamine acts on receptors in the muscle which are blocked by the H_1-receptor antagonist, diphenhydramine.

The mode of action of 5-HT and histamine on *Tupaia* ileum seems to resemble that on human ileum (Fishlock, 1964; Bennett, 1965) and jejunum (Whitney, 1965). Rodent tissues are widely used for analysis of drug action, but for the evaluation of drugs for potential use in man it may be that *Tupaia*, a primitive primate (Schwaier, 1973; 1975), is preferable.

References

BENNETT, A. (1965). A pharmacological investigation of human isolated ileum. *Nature*, **208**, 1289–1291.

BROWNLEE, G. & JOHNSON, E.S. (1963). The site of the 5-hydroxytryptamine receptor on the intramural nervous plexus of the guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.* **21**, 306–322.

DAY, M. & VANE, J.R. (1963). An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **20**, 150–170.

FISHLOCK, D.J. (1964). The action of 5-hydroxytryptamine on the circular muscle of the human ileum and colon *in vitro*. *J. Physiol.*, **170**, 11–12P.

GADDUM, J.H. & HAMEED, K.A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **9**, 240–248.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptors. *Br. J. Pharmac. Chemother.*, **12**, 323–328.

GOODMAN, M. (1966). Phyletic position of tree shrews. *Science, N.Y.*, **153**, 1550.

LE GROS CLARK, W.E. (1971). *The Antecedents of Man.* 3rd edition. *Edinburgh University Press.*

MOORE, W.G. & GOODMAN, M. (1968). A set theoretical approach to immunotoxonomy. Analysis of species comparison in modified Ouchterlony plates. *Bull. Math. Biophys.*, **30**, 279–289.

ROBERTSON, P.A. (1953). An antagonism of 5-hydroxytryptamine by atropine. *J. Physiol.*, **121**, 54P.

ROCHA E SILVA, M., VALLE J.R. & PICARELLI, Z.P. (1953). A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **8**, 378–388.

SCHWAIER, A. (1973). Breeding tupaias (*Tupaia belangeri*) in captivity. *Z. Versuchstierk.*, **15**, 255–271.

SCHWAIER, A. (1975). *Tupaia* low-cost primates for medical research. In *Primate Utilization and Conservation.* ed. Bermant, G. & Lindburg, D.C., pp. 141–150. New York: Wiley.

WHITNEY, B. (1965). A preliminary investigation of the pharmacology of longitudinal muscle strips from human isolated jejunum. *J. Pharm. Pharmac.*, **17**, 465–473.

(*Received October* 17, 1978.
Revised December 5, 1978.)