Br. J. Pharmac. (1986), **89**, 787–792

Presynaptic muscarinic and α-adrenoceptor-mediated regulation of GABA release from myenteric neurones of the guinea-pig small intestine

S. Hashimoto, C. Tanaka[1] & K. Taniyama

Department of Pharmacology, Kobe University School of Medicine, Chuo-ku, Kobe 650, Japan

1 The effects of cholinomimetic and sympathomimetic drugs on the release of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) evoked by high K^+ from the isolated small intestine of the guinea-pig were investigated, in the presence of tetrodotoxin.

2 Acetylcholine and oxotremorine, at concentrations ranging from 10^{-9} to 10^{-6}M inhibited the evoked release of [^{3}H]-GABA in a concentration-dependent manner, while nicotine was without effect. Scopolamine and pirenzepine inhibited the effect of oxotremorine, while hexamethonium had no effect. The IC_{50} values for scopolamine and pirenzepine of the oxotremorine (3×10^{-8}M)-induced inhibition were 1.02×10^{-9}M and 9.78×10^{-10}M, respectively.

3 Noradrenaline, but not isoprenaline inhibited the evoked release of [^{3}H]-GABA. Clonidine (10^{-10}–10^{-6}M) reduced the evoked release of [^{3}H]-GABA in a concentration-dependent manner, but phenylephrine had no effect. The inhibitory effect of clonidine was antagonized by yohimbine but not by prazosin.

4 These findings provide evidence for the localization of M_1- muscarinic and α_2- adrenoceptors on GABAergic nerve terminals and their involvement in the presynaptic control of the release of GABA from the guinea-pig small intestine.

Introduction

Accumulated evidence suggests that γ-aminobutyric acid (GABA) may function as a neurotransmitter in the mammalian enteric nervous system (Tanaka, 1985; Erdö, 1985). High-affinity uptake sites for GABA (Jessen *et al.*, 1979; Krantis & Kerr, 1981; Saffrey *et al.*, 1983), the high concentration of GABA and high activity of glutamate decarboxylase (Taniyama *et al.*, 1982b; Miki *et al.*, 1983), immunohistochemical localization of GABA in the myenteric plexus (Saito *et al.*, 1985; Hills *et al.*, 1985; Saito & Tanaka, 1986) and the neuronal release of GABA evoked by electrical stimulation and high concentrations of potassium (Taniyama *et al.*, 1982a, 1983b; Kerr & Krantis, 1983) all support this hypothesis. The neuronal circuit related to GABAergic neurones has been noted in the enteric nervous system. The target of GABAergic neurones seems to be postganglionic cholinergic neurones, based on the findings that GABA depolarized myenteric neurones apparently contained acetylcholine (ACh) (Cherubini & North, 1984) and that GABA induced a release of ACh and inhibited the stimulation-evoked release of ACh (Taniyama *et al.*, 1983a; Kleinrok & Kilbinger, 1983). One of the inputs to GABAergic neurones may be the neurones which utilize substance P as their transmitter (Tanaka & Taniyama, 1985). The presynaptic GABA autoreceptor also regulates the release of GABA (Taniyama *et al.*, 1985).

Sympathetic and parasympathetic neurones are known to innervate the myenteric plexus and affect motility of the intestine. Therefore, there is a possibility that the GABAergic neurones are influenced by these neurones. In order to elucidate the influence of sympathetic and parasympathetic neurones, the effects of cholinomimetic and sympathomimetic drugs were studied on the release of GABA from myenteric neurones in the guinea-pig ileum.

Methods

Adult guinea-pigs of either sex (300–400 g) were killed by cervical dislocation and 2 cm long strips, 10 cm

[1] Author for Correspondence.

proximal to the ileocaecal sphincter were rapidly prepared.

The methods of incubation and perfusion were as described previously (Taniyama *et al.*, 1983b). The strips were incubated for 1 h at 37°C with [^{3}H]-GABA at a final concentration of 50 nM in oxygenated Krebs solution of the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.19, $NaHCO_3$ 25.0, KH_2PO_4 1.18 and glucose 11. After washing in fresh medium for 30 min, each strip was mounted in an apparatus and superfused with medium at a rate of 1.2 ml min^{-1}. The incubation and superfusion media contained 10^{-5}M aminooxyacetic acid (AOAA) to prevent the metabolism of [^{3}H]-GABA (Baxter & Roberts, 1961) and 10^{-3}M β-alanine to prevent the glial uptake of [^{3}H]-GABA (Schon & Kelly, 1975; Iversen & Kelly, 1975) and gassed with 95% O_2 plus 5% CO_2. The superfusate was collected every 30 s, and the radioactivity measured in a liquid scintillation spectrometer. The high K^+ solution was obtained by isomolar replacement of NaCl by KCl. At the end of the experiments, the tissue was dissolved in Soluene and the radioactivity measured.

The fractional rate of tritium was expressed as the ratio of the amount of tritium released from the tissue during each 30 s collection period to the amount of tritium present in the tissue at the initiation of the corresponding collection period. The tritium content of the tissue at each period was calculated by adding cumulatively the amount of each fractional tritium efflux to the tritium content of the tissue at the end of the experiment. The proportion of unchanged GABA was determined by high-voltage electrophoresis, as described previously (Taniyama *et al.*, 1982a; 1983b). Over 93% of the total radiactivity in the superfusates corresponded to unchanged GABA. Data were analysed by Student's *t* test, and a *P* value of < 0.05 was considered to be statistically significant.

Drugs and chemicals used were as follows: [^{3}H]-GABA (32.8 Ci mmol^{-1}, New England Nuclear, Boston, Mass.), aminooxyacetic acid (AOAA), oxotremorine, noradrenaline bitartrate (NA), isoprenaline hydrochloride and yohimbine hydrochloride (Sigma, St. Louis, MO), ethylene glycol *bis* (β-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), β-alanine, acetylcholine chloride (ACh) and scopolamine hydrobromide (Nakarai Chemicals Ltd, Kyoto, Japan), hexamethonium bromide and clonidine hydrochloride (Tokyo Kasei Co. Ltd, Tokyo, Japan), phenylephrine hydrochloride (American Roland Corp., New York), pirenzepine (Nippon Boehringer Ingelheim Co. Ltd, Hyogo, Japan), tetrodotoxin (Sankyo Co. Ltd, Tokyo, Japan) and prazosin hydrochloride (Taito Pfeizer Co. Ltd, Tokyo, Japan).

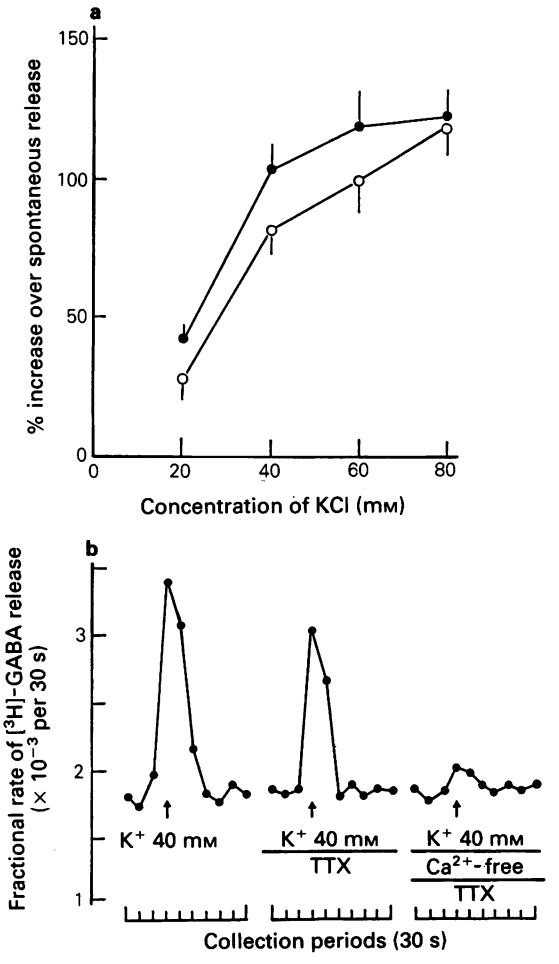

Figure 1 Effects of tetrodotoxin (TTX) and Ca^{2+}-free medium on K^+-evoked [^{3}H]-GABA release. (a) Concentration-response curves for potassium in the absence (●) and presence (○) of 1 μM TTX. (b) K^+ (40 mM)-evoked release of [^{3}H]-GABA in the Ca^{2+}-free medium containing 1 mM EGTA (Ca^{2+}-free). TTX (1 μM) was perfused 20 min before and during the application of KCl for 30 s. Each point represents the mean of 5 preparations and vertical lines show s.e.

Results

Spontaneous and high K^+-evoked release of [^{3}H]-GABA

The experiments were started 60 min after the superfusion, because at this time the spontaneous [^{3}H]-GABA release from strips of guinea-pig ileum preloaded with [^{3}H]-GABA approached a fairly con-

stant level at a fractional rate of $3.39 \pm 0.465 \times 10^{-3}$ min^{-1}. The release of [^{3}H]-GABA evoked by KCl ranging from 20 mM to 80 mM was concentration-dependent and the maximum response was obtained with 60 mM KCl. The KCl-evoked release of [^{3}H]-GABA was partially inhibited by 20 min pretreatment with 10^{-6} M tetrodotoxin (Figure 1a). The [^{3}H]-GABA release evoked by KCl 40 mM was approximately double the spontaneous release and was inhibited by 10^{-6} M tetrodotoxin to approximately 75%. The KCl (40 mM)-evoked [^{3}H]-GABA release in the presence of tetrodotoxin was prevented by perfusion with Ca^{2+}-free medium containing 1 mM EGTA (Figure 1b), indicating that the [^{3}H]-GABA released by KCl had originated from the nerve terminals.

Effects of cholinoceptor agonists and antagonists on high K^+-evoked release of [^{3}H]-GABA

Effects of substances on the [^{3}H]-GABA release evoked by KCl 40 mM were examined in the presence of 10^{-6}M tetrodotoxin. As shown in Figure 2, ACh at concentrations ranging from 10^{-9} to 10^{-6}M inhibited the high K^+-evoked release of [^{3}H]-GABA in a concentration-dependent manner, but did not affect the spontaneous release of [^{3}H]-GABA. The EC_{50} value was 3.22×10^{-8}M. Oxotremorine at 10^{-9}–10^{-6}M, also reduced the high K^+-evoked release of [^{3}H]-GABA in a concentration-dependent manner. The EC_{50} value was 7.40×10^{-9}M. The potency of oxotremorine was 4.33 times that of ACh. Nicotine did not significantly affect the high K^+-evoked release of [^{3}H]-GABA (Figure 2). Perfusion with 10^{-7}M

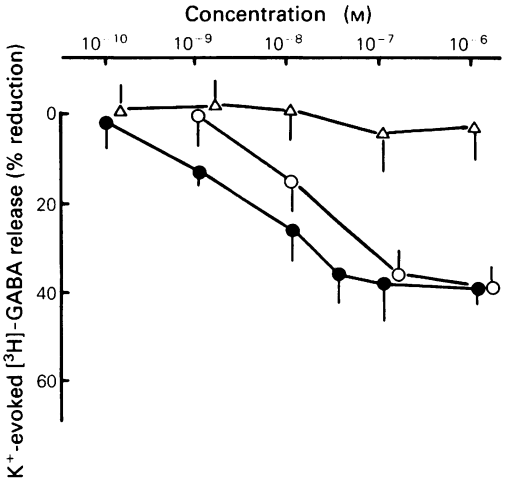

Figure 2 Effects of acetylcholine (○), oxotremorine (●) and nicotine (△) on K^+ (40 mM)-evoked release of [^{3}H]-GABA. Each point is the mean of 5 preparations and vertical lines show s.e.

Table 1 Effects of cholinoceptor agonists on the oxotremorine-induced inhibition of K^+ (40 mM)-evoked release of [^{3}H]-GABA

Drug	*Concentration* (M)	n	*% inhibition of evoked release*
Oxotremorine	3×10^{-8}	28	36.6 ± 9.38*
Oxotremorine + scopolamine	3×10^{-8} + 1×10^{-7}	8	1.2 ± 8.82
Oxotremorine + hexamethonium	3×10^{-8} + 3×10^{-4}	8	34.8 ± 4.38*
Oxotremorine + pirenzepine	3×10^{-8} + 1×10^{-7}	5	2.6 ± 7.85

n = number of experiments. *$P < 0.05$.

scopolamine, but not 3×10^{-4}M hexamethonium antagonized the oxotremorine (3×10^{-8}M)-induced inhibition of the evoked release of [^{3}H]-GABA (Table 1), indicating that the cholinoceptor mediating the inhibition of [^{3}H]-GABA release is muscarinic.

Pirenzepine, an antagonist of the M_1-muscarinic receptor (Hammer *et al.*, 1980), antagonized the oxotremorine-induced inhibition of the evoked [^{3}H]-GABA release (Table 1). When the antagonistic effects of scopolamine and pirenzepine on the oxotremorine

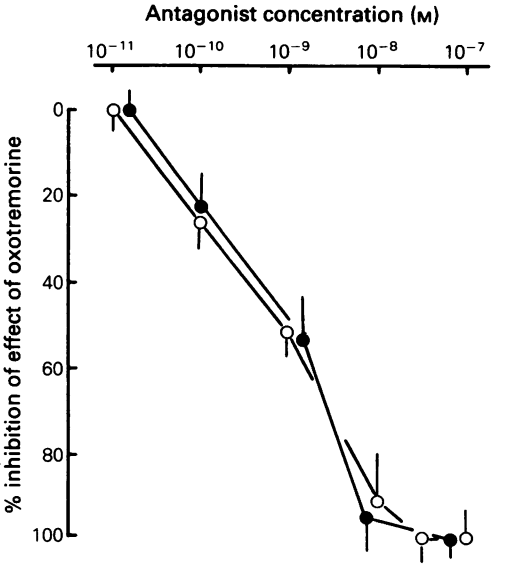

Figure 3 Concentration-dependent inhibition by scopolamine (●) or pirenzepine (○) of oxotremorine-induced inhibition of K^+ (40 mM)-evoked [^{3}H]-GABA release. Oxotremorine (3×10^{-8}M) was applied 3 min before the K^+ stimulation. Each point represents the mean of 5 experiments and vertical lines show s.e.

(3×10^{-8}M)-induced inhibition of high K^+-evoked [^{3}H]-GABA release were compared, almost the same blocking effects were observed (Figure 3). The IC_{50} values for scopolamine and pirenzepine of the oxotremorine (3×10^{-8}M)-induced inhibition were 1.02×10^{-9}M and 9.78×10^{-10}M, respectively.

Effects of adrenoceptor agonists and antagonists on high K^+-evoked release of [^{3}H]-GABA

NA (10^{-7}M), but not isoprenaline (10^{-7}M), inhibited the high K^+-evoked release of [^{3}H]-GABA by 64.3% (Figure 4), suggesting that the receptor involved in the inhibition of the high K^+-evoked release of [^{3}H]-GABA is an α-adrenoceptor. Phenylephrine and clonidine were used to examine whether the effect of NA was mediated via α_1- or α_2- adrenoceptors. As shown in Figure 5, clonidine $10^{-10} - 10^{-6}$M, but not phenylephrine, inhibited the high K^+-evoked [^{3}H]-GABA release in a concentration-dependent manner, and the EC_{50} value of clonidine was 2.52×10^{-10}M. The inhibitory effect of 10^{-7}M clonidine was antagonized by the α_2-adrenoceptor antagonist yohimbine at a concentration of 10^{-7}M, but not by the α_1-adrenoceptor antagonist prazosin (Table 2).

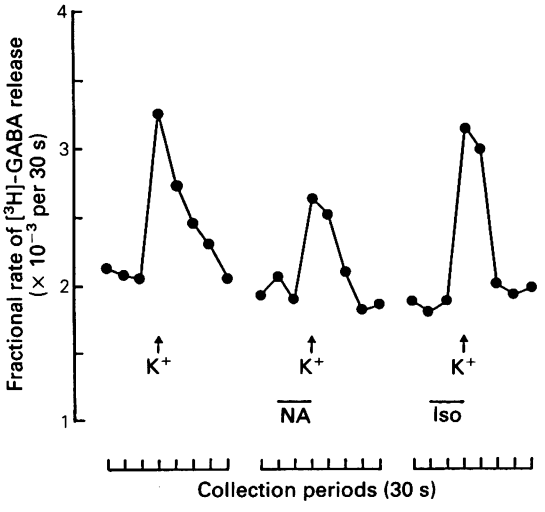

Figure 4 Effects of noradrenaline (NA, 10^{-7}M) and isoprenaline (Iso, 10^{-7}M) on K^+ (40 mM)-evoked [^{3}H]-GABA release. K^+ (40 mM) was applied at arrows for 30 s. Each point represents the mean of 5 experiments.

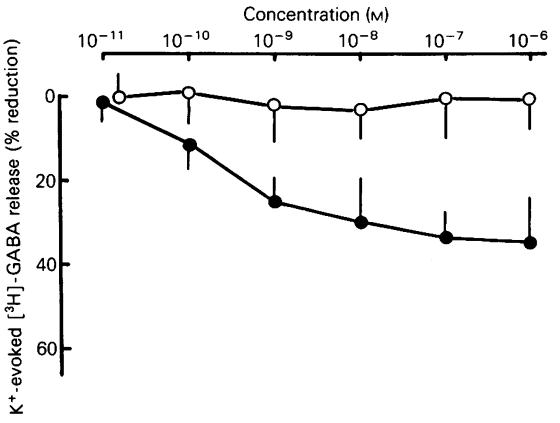

Figure 5 Effects of phenylephrine (○) and clonidine (●) on K^+ (40 mM)-evoked release of [^{3}H]-GABA. Each point is the mean of 5 preparations and vertical lines show s.e.

Table 2 Effects of α-adrenoceptor antagonists on the clonidine-induced inhibition of K^+ (40 mM)-evoked [^{3}H]-GABA release

Drug	*Concentration* (M)	n	*% inhibition of evoked release*
Clonidine	10^{-7}	10	33.6 ± 8.61*
Clonidine + prazosin	10^{-7} 10^{-6}	5	30.1 ± 8.95*
Clonidine + yohimbine	10^{-7} 10^{-7}	5	−1.8 ± 12.9

n = number of experiments. *$P < 0.05$.

Discussion

Evidence was obtained to support the proposal that cholinoceptors and adrenoceptors are involved in the presynaptic regulation of GABA release. The effects of cholinomimetic and sympathomimetic drugs were examined with regard to the Ca^{2+}-dependent release of GABA evoked by high K^+ in the presence of tetrodotoxin. This approach has been used to investigate presynaptic modulation of neurotransmitter release (Starke, 1981), because tetrodotoxin blocks neuronal conduction without affecting direct depolarization of the nerve terminals (Narahashi, 1974).

ACh reduced the high K^+-evoked release of GABA in a concentration-dependent manner. Oxotremorine but not nicotine caused a concentration-dependent reduction of high K^+-evoked GABA release and the

inhibitory effect of oxotremorine was antagonized by scopolamine but not by hexamethonium. These findings indicate that it is the muscarinic receptors which are involved in the ACh-mediated inhibition of [^{3}H]-GABA release. The heterogeneity of muscarinic receptors, M_1-and M_2-types has been described (Brown *et al.*, 1980; Watson *et al.*, 1982; Birdsall & Hulme, 1983). Scopolamine appears to show virtually the same affinity for muscarinic receptor subtypes, while pirenzepine seems to differentiate between high (M_1)-and low (M_2)-affinity subtypes of muscarinic receptor (Hammer *et al.*, 1980). Scopolamine and pirenzepine, both muscarinic receptor antagonists antagonized the oxotremorine-induced inhibition of GABA release with much the same potency. These results indicate that pirenzepine-sensitive muscarinic receptors (M_1) may be involved in the presynaptic regulation of GABA release from enteric GABAergic neurones. Pirenzepine-sensitive muscarinic receptors have been linked to the regulation of secretion of gastric somatostatin and gastrin (Sue *et al.*, 1985) and to the potentiation of dopamine release from the rat nucleus accumbens, cortex and hippocampus (Raiteri *et al.*, 1984; De Belleroche & Gardiner, 1985). The muscarinic autoreceptors located on the cholinergic nerve terminals are considered to be the M_2-type (Kilbinger & Nafziger, 1985; Meyer & Otero, 1985). However, the muscarinic receptor which regulates the release of GABA is the M_1-type and differs from the autoreceptor on the cholinergic nerve terminals.

NA reduced the high K^+-evoked release of GABA, but isoprenaline had no effect, thereby indicating that α-adrenoceptors are involved in the inhibition of GABA release. Clonidine, an α_2-adrenoceptor agonist, but not phenylephrine an α_1-adrenoceptor agonist inhibited the evoked release of GABA. The inhibitory effect of clonidine was antagonized by yohimbine, an α_2-adrenoceptor antagonist, but not by prazosin, an α_1-adrenoceptor antagonist. Therefore, NA probably inhibits the evoked release of GABA via α_2-adrenoceptors. Presynaptic α_2-adrenoceptors have been found to exist on adrenergic and cholinergic nerve terminals (Vizi, 1979; Kilbinger & Wessler, 1979; Tanaka & Starke, 1979) in the enteric nervous systems. All these results support the proposal that both M_1-muscarinic receptors and α_2-adrenoceptors are located on the enteric GABAergic nerve terminals and regulate the release of GABA.

GABA regulates ACh release through $GABA_A$- and $GABA_B$-receptors located on postganglionic cholinergic neurones (Taniyama *et al.*, 1983a; Kleinrok & Kilbinger, 1983). The present results indicate that a short neuronal circuit, GABAergic-cholinergic and cholinergic-GABAergic circuits may be present in the intestine and involved in the regulation of the motility of the intestine. Most adrenergic neurones which innervate the intestine form a terminal network around the myenteric ganglia (Jacowitz, 1965; Costa & Gabella, 1971). NA released from adrenergic nerve terminals within the myenteric plexus inhibits presynaptically the release of ACh through α_2-adrenoceptors (Wikberg & Lefkowitz, 1982; Alberts & Stjärne, 1982). Therefore, the possibility that adrenergic neurones regulate the release of ACh from cholinergic neurones, via GABAergic neurones, warrants further attention.

This work was supported by grants from The Ministry of Education, Science and Culture, Japan. We thank M. Ohara of Kyushu University for comments on the manuscript.

References

ALBERTS, P. & STJÄRNE, L. (1982). Facilitation, and muscarinic and α-adrenergic inhibition of the secretion of ^{3}H-acetylcholine and ^{3}H-noradrenline from guinea-pig ileum myenteric nerve terminals. *Acta physiol. scand.*, **116**, 83–92.

BAXTER, C.F. & ROBERTS, E. (1961). Elevation of γ-aminobutyric acid in brain: selective inhibition of γ-aminobutyric-α-ketoglutaric acid transaminase. *J. biol. Chem.*, **236**, 3287–3294.

BIRDSALL, N.J.M. & HULME, E.C. (1983). Muscarinic receptor subclasses. *Trends Pharmac. Sci.*, **4**, 459–463.

BROWN, D.A., FORWARD, A. & MARSH, S. (1980). Antagonist discrimination between ganglionic and ileal muscarinic receptors. *Br. J. Pharmac.*, **71**, 362–364.

CHERUBINI, E. & NORTH, R.A. (1984). Actions of γ-aminobutyric acid on neurones of guinea-pig myenteric plexus. *Br. J. Pharmac.*, **82**, 93–100.

COSTA, M. & GABELLA, G. (1971). Adrenergic innervation of the alimentary canal. *Z. Zellforsch.*, **122**, 357–377.

DE BELLEROCHE, J. & GARDINER, I.M. (1985). Muscarinic receptors discriminated by pirenzepine are involved in the regulation of neurotransmitter release in rat nucleus accumbens. *Br. J. Pharmac.*, **86**, 505–508.

ERDÖ, S.L. (1985). Peripheral GABAergic mechanisms. *Trends Pharmac Sci.*, **6**, 205–208.

HAMMER, R., BERRIE, C.P., BIRDSALL, N.J.M., BURGEN, A.S.V. & HULME, E.C. (1980). Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature*, **283**, 90–92.

HILLS, J.M., JESSEN, K.R. & SAFFREY, M.J. (1985). Immunohistochemical evidence for GABAergic neurones in the enteric nervous system of the rat and guinea-pig. *J. Physiol.*, **367**, 55p.

IVERSEN, L.L. & KELLY, J.S. (1975). Uptake and metabolism of γ-aminobutyric acid by neurons and glial cells. *Biochem. Pharmac.*, **24**, 933–938.

JACOBOWITZ, D. (1965). Histochemical studies of the autonomic innervation of the gut. *J. Pharmac. exp. Ther.*, **149**,

358–364.

JESSEN, K.R., MIRSKY, R., DENNISON, M.E. & BURNSTOCK, G. (1979). GABA may be a neurotransmitter in the vertebrate peripheral nervous system. *Nature*, **281**, 71–74.

KERR, D.I.B. & KRANTIS, A. (1983). Uptake and stimulus-evoked release of [^{3}H]-γ-aminobutyric acid by myenteric nerves of guinea-pig intestine. *Br. J. Pharmac.*, **78**, 271–276.

KILBINGER, H. & NAFZIGER, M. (1985). Two types of neuronal muscarine receptors modulating acetylcholine release from guinea-pig myenteric plexus. *Naunyn-Schmiedebergs Arch. Pharmac.*, **328**, 304–309.

KILBINGER, H. & WESSLER, I. (1979). Increase by α-adrenergic drugs of acetylcholine release evoked by field stimulation of the guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmac.*, **309**, 255–257.

KLEINROK, A. & KILBINGER, H. (1983). γ-Aminobutyric acid and cholinergic transmission in the guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmac.*, **322**, 216–220.

KRANTIS, A. & KERR, D.I.B. (1981). Autoradiographic localization of [^{3}H]-gamma-aminobutyric acid in the myenteric plexus of the guinea-pig small intestine. *Neurosci. Lett.*, **23**, 263–268.

MEYER, E.M. & OTERO, D.H. (1985). Pharmacological and ionic characterizations of the muscarinic receptors modulating [^{3}H]acetylcholine release from rat cortical synaptosomes. *J. Neurosci.*, **5**, 1202–1207.

MIKI, Y., TANIYAMA, K., TANAKA, C. & TOBE, T. (1983). GABA, glutamic acid decarboxylase and GABA transaminase levels in the myenteric plexus in the intestine of humans and other mammals. *J. Neurochem.*, **40**, 861–865.

NARAHASHI, T. (1974). Chemicals as tools in the study of excitable membranes. *Physiol. Rev.*, **54**, 813–889.

RAITERI, M., LEARDI, R. & MARCHI, M. (1984). Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain. *J. Pharmac. exp. Ther.*, **228**, 209–214.

SAFFREY, M.J., MARCUS, N., JESSEN, K.R. & BURNSTOCK, G. (1983). Distribution of neurons with high-affinity uptake sites for GABA in the myenteric plexus of the guinea-pig, rat and chicken. *Cell Tissue Res.*, **234**, 231–235.

SAITO, N., KUMOI, K., SAKAUE, M., KONDO, M. & TANAKA, C. (1985). Immunohistochemical study for GABA and glutamate containing neurons. *Jap. J. Pharmac.*, **39**, 74P.

SAITO, N. & TANAKA, C. (1986). Immunohistochemical demonstration of GABA containing neurons in the guinea-pig ileum using purified GABA antiserum. *Brain Res.*, **376**, 78–84.

SCHON, F. & KELLY, J.S. (1975). Selective uptake of [^{3}H]ß-alanine by glia: association with the glial uptake system for GABA. *Brain Res.*, **86**, 243–257.

STARKE, K. (1981). Presynaptic receptors. *A. Rev. Pharmac. Tox.*, **21**, 7–30.

SUE, R., TOOMEY, M.L., TODISCO, A., SOLL, A.H. & YAMADA, T. (1985). Pirenzepine-sensitive muscarinic receptors regulate gastric somatostatin and gastrin. *Am. J. Physiol.*, **248**, G184–G187.

TANAKA, C. (1985). γ-Aminobutyric acid in peripheral tissues. *Life Sci.*, **37**, 2221–2235.

TANAKA, C. & TANIYAMA, K. (1985). Substance P provoked γ-aminobutyric acid release from myenteric plexus of the guinea-pig small intestine. *J. Physiol.*, **362**, 319–329.

TANAKA, T. & STARKE, K. (1979). Binding of ^{3}H-clonidine to an α-adrenoceptor in membranes of guinea-pig ileum. *Naunyn-Schmiedebergs Arch. Pharmac.*, **309**, 207–215.

TANIYAMA, K., HANADA, S. & TANAKA, C. (1985). Autoreceptors regulate γ-[^{3}H] aminobutyric acid release from the guinea pig small intestine. *Neurosci. Lett.*, **55**, 245–248.

TANIYAMA, K., KUSUNOKI, M., SAITO, N. & TANAKA, C. (1982a). Release of γ-aminobutyric acid from cat colon. *Science*, **217**, 1038–1040.

TANIYAMA, K., KUSUNOKI, M., SAITO, N. & TANAKA, C. (1983a). GABA evoked ACh release from isolated guinea pig ileum. *Life Sci.*, **32**, 2349–2353.

TANIYAMA, K., MIKI, Y., KUSUNOKI, M., SAITO, N. & TANAKA, C. (1983b). Release of endogenous and labeled GABA from isolated guinea-pig ileum. *Am. J. Physiol.*, **245**, G717–G721.

TANIYAMA, K., MIKI, Y. & TANAKA, C. (1982b). Presence of γ-aminobutyric acid and glutamic acid decarboxylase in Auerbach's plexus of cat colon. *Neurosci. Lett.*, **29**, 53–56.

VIZI, E.S. (1979). Presynaptic modulation of neurochemical transmission. *Prog. Neurobiol.*, **12**, 181–290.

WATSON, M., ROESKE, W.R. & YAMAMURA, H.I. (1982). [^{3}H] pirenzepine selectively identifies a high affinity population of muscarinic cholinergic receptors in the rat cerebral cortex. *Life Sci.*, **31**, 2019–2023.

WIKBERG, J.E.S. & LEFKOWITZ, R.J. (1982). Alpha$_2$ adrenergic receptors are located prejunctionally in the Auerbach's plexus of the guinea-pig small intestine: Direct demonstration by radioligand binding. *Life Sci.*, **31**, 2899-2905.

(Received May 13, 1986.
Revised July 23, 1986.
accepted July 31, 1986.)