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Stimulation of receptors of γ-aminobutyric acid modulates the release of cholecystokinin-like immunoreactivity from slices of rat neostriatum

Ute Conzelmann, Dieter K. Meyer[1] & Günter Sperk*

Department of Pharmacology, University of Freiburg, D-7800 Freiburg, F.R.G. and Department of Pharmacology*, University of Innsbruck, A-6020 Innsbruck, Austria

1 Slices of rat dorsal neostriatum were incubated in Krebs-Henseleit medium and the release of cholecystokinin-like immunoreactivity (CCK-IR) was induced by veratridine or high concentrations of K^+. It was investigated whether drugs which act at receptors for γ-aminobutyric acid (GABA) affected the release.

2 The $GABA_A$-receptor agonists muscimol and isoguvacine enhanced the veratridine-induced release of CCK-IR. This effect was abolished by the $GABA_A$-receptor antagonist, bicuculline. When used alone, bicuculline decreased the release.

3 The $GABA_B$-receptor agonist, (−)-baclofen, decreased the veratridine-induced release of CCK-IR. The stereoisomer (+)-baclofen, which has low intrinsic activity, had no effect when used alone, but antagonized the effect of (−)-baclofen as did δ-amino-*n*-valeric acid, another antagonist at $GABA_B$-receptors.

4 When the release of CCK-IR was stimulated by K^+ (40 mM) in the presence of tetrodotoxin, it was no longer affected by $GABA_A$-receptor agonists or antagonists. Thus, their sites of action were probably not in the immediate vicinity of the nerve-endings which release CCK-IR.

5 Under these conditions, stimulation of $GABA_B$-receptors still reduced the release of CCK-IR. Therefore, it is concluded that these receptors are in the immediate vicinity of or even on the terminals which release CCK-IR.

Introduction

The octapeptide cholecystokinin-sulphate is present in neuronal afferents in rat caudatoputamen (Hökfelt *et al.*, 1980; Meyer *et al.*, 1982). When incubated *in vitro*, slices of rat dorsal caudatoputamen release cholecystokinin-immunoreactivity (CCK-IR) upon stimulation with veratridine or high concentrations of K^+. This stimulus-induced secretion is Ca^{2+}-dependent (Meyer & Krauss, 1983; Conzelmann *et al.*, 1984). It can be modulated by stimulation of dopamine (D) receptors. Activation of D_2-receptors enhances, while stimulation of D_1-receptors diminishes it (Meyer & Krauss, 1983). Both these actions of dopamine are probably not direct, but are mediated by striatal neuronal elements (Conzelmann *et al.*, 1984).

More than 90% of the neurones of rat striatum have been described as the medium size spiny type. They are efferent neurones which project to the globus pallidus and the substantia nigra (Ribak *et al.*, 1979; Somogyi & Smith, 1979). Their dendrites and perikarya form synapses with dopamine-containing afferents (Freund *et al.*, 1984). Substance P (Bolam *et al.*, 1983), enkephalin (Pickel *et al.*, 1980) and dynorphin (Zamir *et al.*, 1984) as well as GABA (Ribak *et al.*, 1979; Somogyi & Smith, 1979) have been found in subpopulations of these neurones.

The high concentration of GABA in rat caudatoputamen seems to be due to its synthesis in axon collaterals of medium size spiny neurones as well as in a smaller number of intrinsic neurones (Ribak *et al.*, 1979).

Thus, GABA-neurones could participate in the regulation of the release of CCK-IR from its nerve endings in the caudatoputamen. In a first step to study these possible interactions, we have investigated the effects of some GABA-receptor agonists and antagonists on the stimulus-induced release of CCK-IR.

Since stimulation of GABA-receptors turned out to modulate the veratridine-induced release of CCK-IR,

[1]Author for correspondence

some additional experiments were performed to learn more about their location. For this purpose, the effects of GABA-agonists were studied on the release of CCK-IR as induced by K^+ (40 mM). Tetrodotoxin (5×10^{-7} M) was added to block neuronal Na^+-channels and in this way to inhibit current propagation. Since under these conditions neuronal release is only due to the direct action of K^+ on the transmitter releasing nerve terminals, it was possible to decide whether the GABA-receptors which modulated the release of CCK-IR were located in the immediate vicinity of the CCK-terminals.

Methods

Preparation of slices of caudatoputamen

Female Wistar rats (200–250 g) were used. They were housed in diurnal lighting conditions (light on 06 h 00 min – off 18 h 00 min) and given food and water *ad libitum*.

Slices of dorsal caudatoputamen were prepared as described previously (Meyer & Krauss, 1983). The rats were decapitated and the brains were quickly removed. The dorsal caudatoputamen was dissected on a cooled plate (0°) by two coronal cuts. Care was taken to remove the nucleus accumbens, the ventral neostriatum plus claustrum and any cortical tissue. Horizontal slices (0.3 mm) were cut with a McIlwain chopper.

Incubation procedure

The slices were pooled, incubated for 60 min in 2 ml incubation medium (composition see below) and then distributed into plastic tubes (2 slices per tube). The incubation medium was collected every 20 min for determination of CCK-IR. During the second and third collection period the spontaneous release was usually below the detection limit of our radioimmunoassay. During the fourth period, the release of CCK-IR was stimulated for 20 min by adding veratridine (3.75×10^{-6} M) or by increasing the concentration of K^+ in the medium (isotonicity was maintained by reducing the concentration of NaCl). In pilot experiments which are not shown here it had been established that none of the agents used affected the spontaneous release. Therefore, all drugs were present only during the period of incubation. After the experimental (fourth) period the slices were blotted and weighed. The incubation medium was composed of (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.11, bacitracin 0.02 and gelatine 0.1%. The medium was bubbled with a mixture of 5% CO_2/95% O_2. Incubation volume was 1 ml.

Determination of cholecystokinin-immunoreactivity

The radioimmunoassay used has been described previously (Beinfeld *et al.*, 1981). The antiserum was a gift of M.C. Beinfeld, St Louis, U.S.A. ^{125}I gastrin, obtained from Amersham, Braunschweig, was used as the label. Sulphated cholecystokinin$_8$ (a gift of S.J. Lucania, Squibb, Princeton, N.J., U.S.A.) was used as standard. The range of determination of the assay was from 1.5 to 50.0 pg. A 0.3 ml aliquot of the incubation medium was used for determination of CCK-IR.

Sample analysis by high pressure liquid chromatography

Incubation media of striatal slices which had been stimulated with veratridine (7.5×10^{-6} M) or with K^+ (55 mM) were lyophilized and redissolved in a small volume (300 µl) of 2 N acetic acid. Thirty µl aliquots were directly measured in the radioimmunoassay to establish the content of CCK-IR of these samples. Larger aliquots (200 µl) were injected into a 'Hi pore' reversed phase column (250 × 4.6, Biorad), which was eluted with a 0.15 M triethylamine formiate buffer, pH 6.5, at a flow rate of 0.75 ml min^{-1}. After eluting isocratically for 5 min, a gradient with linearly increasing concentrations of acetonitrile (0 to 50%) was applied for the following 30 min. The procedure was concluded by an isocratical elution for 5 min (triethylamine formiate buffer 50%/acetonitrile 50%). Fractions (0.75 ml) were collected, twice lyophilized and subjected to radioimmunoassay.

Calculation of data and statistical evaluation

Within one experiment, controls (slices treated with veratridine or high K^+ only) were always compared with slices treated with different concentrations of the respective drugs. For experimental reasons the averages of the absolute values for identical treatments slightly differed from one experiment to the other. When absolute values of corresponding experiments were averaged, their s.e.mean became large and sometimes masked effects of drug treatments. Therefore, the stimulus-induced release is expressed as a percentage of controls. The absolute values of these controls are given in the legend of the figures. For this purpose, stimulus-induced release was calculated by subtracting the spontaneous release of the third period (if this had been measurable at all) from the stimulated release of the fourth period. Within one set of experiments, these net releases of CCK-IR induced by the respective stimulus alone were averaged and taken as 100%.

Values are given as $x \pm$ s.e.mean (n). One-way analysis of variance followed by Bartlett's test and Student's t test were used for statistical evaluation.

Drugs

The following were used: (−)-bicuculline methiodide, γ-aminobutyric acid, picrotoxin, tetrodotoxin, δ-amino-*n*-valeric acid and (±)-nipecotate purchased from Sigma Chemical Co. (München, FRG), isoguvacine-HBr from Research Biochemicals Incorporated (Boston, U.S.A.). (+)- and (−)-baclofen were gifts from Ciba-Geigy (Basel, Switzerland). Acetonitrile (Merck, Darmstadt) was h.p.l.c. grade; all other substances were reagent grade.

Results

Analysis of incubation media by high pressure liquid chromatography

CCK-IR was recovered from the reverse phase column with a typical elution profile (Figure 1). Cholecystokinin$_8$-sulphate standard (upper panel) and the CCK-IR contained in incubation medium after stimulation with veratridine (7.5×10^{-6} M; middle panel) or after stimulation with K^+ (55 mM; lower panel) showed the same pattern. In fraction 5, the solvent front was usually observed, while the immunoreactivity of the standard as well as of the test samples was found only in the fractions shown. It eluted mainly in fractions 30 and 31. Seventy-three and 88% of the immunoreactivity contained in the samples stimulated with K^+ or veratridine, respectively, could be recovered, indicating that the immunoreactivity measured was indeed due to cholecystokinin$_8$-sulphate.

Effects of $GABA_A$-receptor stimulation on the veratridine-induced release of CCK-IR

The $GABA_A$-agonist muscimol (Johnston, 1978; Bowery *et al.*, 1983) significantly enhanced the veratridine-induced release of CCK-IR, when used at concentrations of 10^{-6} M and 10^{-5} M (Figure 2). The $GABA_A$-receptor antagonist bicuculline (5×10^{-6} M) (Curtis *et al.*, 1970) prevented this effect of muscimol (10^{-6} and 10^{-5} M; Figure 2). When used alone, bicuculline caused a concentration-dependent decrease of the veratridine-induced release of CCK-IR (Figure 2).

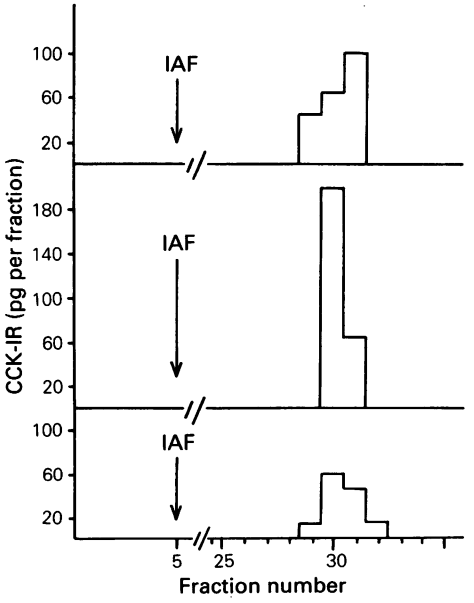

Figure 1 Elution pattern of cholecystokinin$_8$-sulphate (upper panel) and incubation media of striatal slices after stimulation with veratridine (7.5×10^{-6} M; middle panel) or K^+ (55 mM; lower panel). The ordinate scale shows immunoreactivity found per fraction, abscissa scale shows fraction number; the solvent front is indicated by the arrow. Amounts of cholecystokinin$_8$-sulphate large enough to be detected by photometric methods also eluted in fractions 30 and 31.

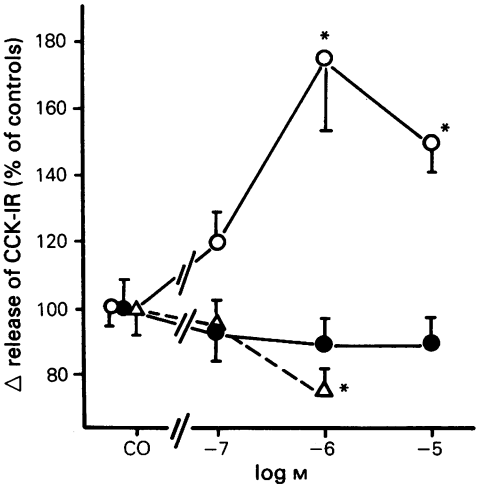

Figure 2 Effects of muscimol (○), muscimol in the presence of bicuculline (5×10^{-6} M; ●) and bicuculline (△) on the veratridine-induced release of cholecystokinin-like immunoreactivity (CCK-IR). Ordinate scale: release of CCK-IR as % of controls (calculation see methods). Abscissa scale: drug concentrations. The absolute values of the controls (pg CCK-IR mg^{-1} w.w.; x ± s.e.mean) and the range of *n* values of the respective experiments were as follows: muscimol (1.6 ± 0.5; $n = 5-9$); bicuculline (1.6 ± 0.3; $n = 5-9$); muscimol in the presence of a constant concentration of bicuculline (1.4 ± 0.5; $n = 5-6$). $^*P < 0.05$ as compared to the respective control.

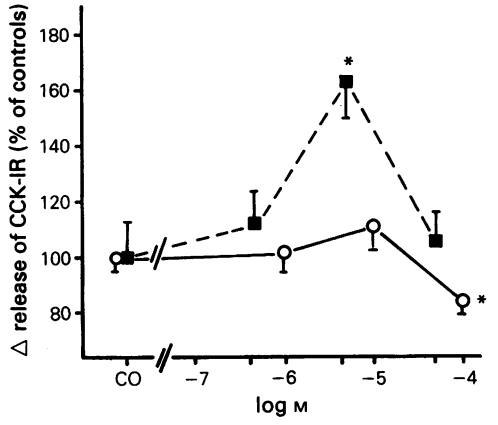

Figure 3 Effects of isoguvacine (■) and GABA in the presence of (±)-nipecotate 5×10^{-4} M (○) on the veratridine-induced release of cholecystokinin-like immunoreactivity (CCK-IR). The absolute values of the controls (pg CCK-IR mg^{-1} w.w.; x ± s.e.mean) and the range of n values of the respective experiments were as follows: isoguvacine (3.2 ± 0.5; $n = 5$–6); GABA (5.5 ± 0.3; $n = 9$–14). $^*P < 0.05$ as compared to the respective control. For further explanation see legend of Figure 1.

A bell-shaped concentration-response curve was observed with isoguvacine: the selective $GABA_A$-receptor agonist (Krogsgaard-Larsen *et al.*, 1977) significantly enhanced the release of CCK-IR at a concentration of 5×10^{-6} M, but not at 5×10^{-5} M (Figure 3).

Effects of $GABA_B$-receptor stimulation on the veratridine-induced release of CCK-IR

The $GABA_B$-receptor agonist, (−)-baclofen (10^{-3} M; Bowery *et al.*, 1980; Hill & Bowery, 1981) significantly decreased the veratridine-induced release of CCK-IR (Table 1), while (+)-baclofen, the respective stereoisomer, had no significant effect (Table 1). In the presence of equimolar concentrations of (+)-baclofen, (−)-baclofen no longer had a significant effect on the release of CCK-IR (Table 1). Also the $GABA_B$-receptor antagonist δ-amino-*n*-valeric acid (10^{-3} M; Muhyaddin *et al.*, 1982) blocked the effect of (−)-baclofen. δ-Amino-*n*-valeric acid did not have an effect when used alone (data not shown).

Effects of GABA on the veratridine-induced release of CCK-IR

In the presence of (±)-nipecotate (5 ± 10^{-4} M) which blocks the uptake of GABA into neuronal and glial elements (Johnston *et al.*, 1976; Shousboe *et al.*, 1979), GABA (10^{-6} and 10^{-5} M) had no significant effect on the veratridine-induced release of CCK-IR. When used with a concentration of 10^{-4} M, however, GABA significantly decreased the release (Figure 3).

Table 1 Effects of agents, which act on $GABA_B$-receptors, on the veratridine-induced release of cholecystokinin-like immunoreactivity (CCK-IR, given as % of controls)

	0	10^{-5} M	10^{-4} M	10^{-3} M
(−)-Baclofen	100 ± 6	108 ± 5	91 ± 5	80 ± 5*
(+)-Baclofen	100 ± 7	86 ± 15	118 ± 12	120 ± 9
(−)-Baclofen plus (+)-Baclofen	100 ± 8		120 ± 7	95 ± 3
(−)-Baclofen plus δ-amino-*n*-valeric acid (10^{-3} M)	± 4		92 ± 8	101 ± 10

The absolute values of the controls (pg CCK-IR mg^{-1} w.w.; x ± s.e.mean) and the range of n values of the respective experiments were as follows: (−)-baclofen (5.6 ± 0.6; $n = 4$–6); (+)-baclofen (4.3 ± 0.5; $n = 5$–8); (−)- plus (+)-baclofen (5.8 ± 0.7; $n = 4$), both agents were used in equimolar concentrations; (−)-baclofen plus δ-amino-*n*-valeric acid (4.5 ± 0.2; $n = 6$).

$^*P < 0.05$ as compared to the respective control. For further explanation see legend of Figure 1.

Table 2 Effects of agents, which act on $GABA_A$-receptors, on the release of cholecystokinin-like immunoreactivity (CCK-IR) as induced by K^+ (40 mM) in the presence of tetrodotoxin (5×10^{-7} M) (CCK-IR is given as % of controls)

	0	10^{-6} M	10^{-5} M	10^{-4} M
Muscimol	100 ± 9	107 ± 6	102 ± 7	
Picrotoxin	100 ± 8	106 ± 12	94 ± 10	97 ± 8
Bicuculline	100 ± 6	108 ± 9	98 ± 8	102 ± 9
GABA plus nipecotate (5×10^{-4} M)	100 ± 7	106 ± 5	73 ± 7*	77 ± 7*

The absolute values of the controls (pg CCK-IR mg^{-1} w.w.; x ± s.e.mean) and the range of n values of the respective experiments were as follows: muscimol (6.7 ± 0.9; $n = 5-6$), picrotoxin (5.4 ± 0.7; $n = 6$), bicuculline (4.5 ± 0.4; $n = 5-6$), GABA plus (±)-nipecotate (5.9 ± 0.7; $n = 6-7$). $^*P < 0.05$ as compared to the respective control. For further explanation see legend of Figure 1.

Effects of GABA on the release of CCK-IR as stimulated by K^+ (40 mM) in the presence of tetrodotoxin (5×10^{-7} M)

When the release of CCK-IR was stimulated by K^+ (40 mM) in the presence of tetrodotoxin (5×10^{-7} M), it was not changed by muscimol (10^{-6} and 10^{-5} M; Table 2). Neither blockade of the $GABA_A$-receptor-ionophore by picrotoxin (10^{-6} to 10^{-4} M; Takeuchi & Takeuchi, 1969; Ticku *et al.*, 1978) nor the $GABA_A$-receptor antagonist bicuculline (10^{-6} and 10^{-5} M) affected the release of CCK-IR (Table 2) indicating that $GABA_A$-receptors no longer modulated the release. However, in the presence of (±)-nipecotate (5×10^{-4} M), GABA (10^{-5} and 10^{-4} M) significantly reduced the release (Figure 4) indicating that it stimulated receptors of the $GABA_B$-type which mediated this effect.

This assumption was confirmed by the observation that the inhibitory effect of GABA (10^{-4} M) on the release of CCK-IR was not affected by picrotoxin (10^{-5} M; Figure 4a), but was antagonized by δ-amino-*n*-valeric acid (Figure 4b), an antagonist at $GABA_B$-receptors.

Effect of baclofen stereoisomers on the release of CCK-IR as stimulated by K^+ (50 mM) in the presence of tetrodotoxin (5×10^{-7} M)

(–)-Baclofen (10^{-6} to 10^{-4} M) significantly decreased the release of CCK-IR, while (+)-baclofen had no effect, when used in the same concentrations (Figure 5). In the presence of δ-amino-*n*-valeric acid (10^{-3} M), which did not affect the release of CCK-IR when used alone (data not shown), the decrease caused by (−)-baclofen (10^{-5} M) was no longer observed (Figure 5).

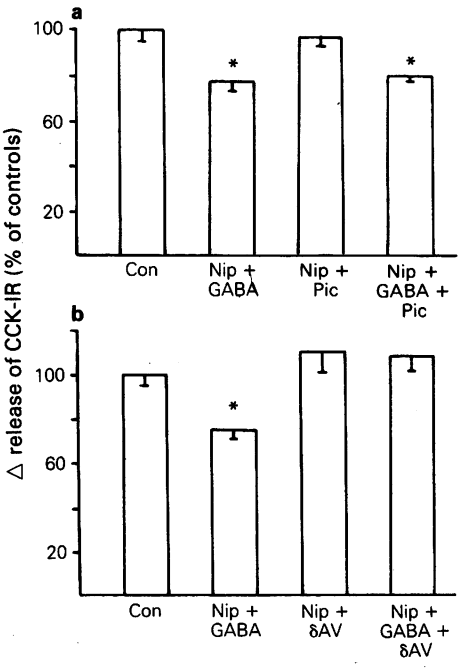

Figure 4 Effects of GABA and GABA-receptor antagonists on the release of cholecystokinin-like immunoreactivity (CCK-IR) as induced by K^+ (40 mM) in the presence of tetrodotoxin (5×10^{-7} M). (±)-Nipecotate (5×10^{-4} M Nip); picrotoxin (10^{-5} M; Pic); δ-aminovaleric acid (10^{-3} M; AV). The absolute values of the controls (pg CCK-IR mg^{-1} w.w.; x ± s.e.mean) and the range of n-values of the respective experiments were as follows: (a) (7.9 ± 0.7; $n = 6$); (b) (7.9 ± 0.4; $n = 6$). $^*P < 0.05$ as compared to the respective control. For further explanation see legend of Figure 1.

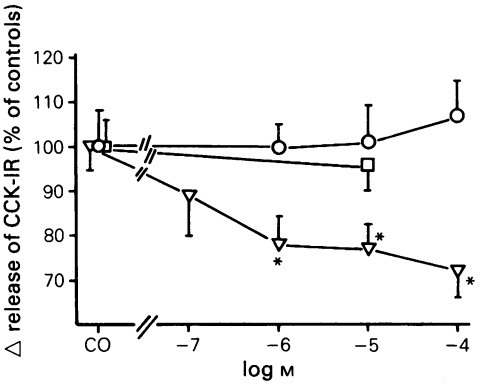

Figure 5 Effects of (−)-baclofen (▽), (+)-baclofen (○) and (−)-baclofen in the presence of δ-amino-*n*-valeric acid (10^{-3} M) (□) on the release of cholecystokinin-like immunoreactivity (CCK-IR) as induced by K^+ (40 mM) in the presence of tetrodotoxin (5×10^{-7} M). The absolute values of the controls (pg CCK-IR mg^{-1} w.w.; x ± s.e.mean) and the range of *n* values of the respective experiments were as follows: (−)-baclofen (5.4 ± 0.6; $n = 6$); (+)-baclofen (5.0 ± 0.5; $n = 5-6$); (−)-baclofen plus δ-amino-*n*-valeric acid (4.4 ± 0.5; $n = 7$). $^*P < 0.05$ as compared to the respective control. For further explanation see legend of Figure 1.

Discussion

Stimulation of slices of rat caudatoputamen with veratridine or high concentrations of K^+ caused the release of immunoreactive material which co-eluted with cholecystokinin$_8$-sulphate, when tested by high pressure liquid chromatography (Figure 1). Thus, it seems appropriate to use the term cholecystokinin-like immunoreactivity (CCK-IR) for the substance released from striatal slices. As already observed in previous studies (Meyer & Krauss, 1983; Conzelmann *et al.*, 1984) spontaneous release was too low to be measured consistently and was not affected by the agents used in this study to modulate the stimulated release of CCK-IR.

Stimulation of GABA-receptors of the A- and B-type changed the veratridine-induced release of CCK-IR from rat striatal slices: the $GABA_A$-receptor agonists muscimol and isoguvacine enhanced the release, while the $GABA_B$-agonist (−)-baclofen reduced it. The effects of the $GABA_A$- and $GABA_B$-agonists were antagonized by the respective antagonists.

Since stimulation of $GABA_A$-receptors is associated with inhibition of neuronal activity, it seems surprising that it enhanced the release of CCK-IR. However, our observation is not without precedence in the striatum. Thus, $GABA_A$-receptor activation increases the stimulus-induced release of glutamate (Mitchell, 1980) which like CCK is a transmitter found in cortical afferents (for review, see McGeer *et al.*, 1979). Our findings obtained with the K^+ (40 mM)/ tetrodotoxin stimulation indicate that the $GABA_A$-agonists did not act directly on the CCK-containing nerve endings which extend into the striatum, since the agonist muscimol as well as the antagonists picrotoxin and bicuculline were inactive under these circumstances. Thus, the $GABA_A$-receptor-mediated increase in the release of CCK-IR may be due to an indirect action, possibly to the inhibition of neuronal elements which inhibit the release of CCK-IR. This explanation is tempting in view of reports on $GABA_A$-receptor-mediated reductions in the release of enkephalin-like immunoreactivity from rat striatal slices (Osborne & Herz, 1982; Harsing *et al.*, 1982). Enkephalins are generally associated with inhibition of neuronal activity (Nicoll *et al.*, 1980). However, enkephalins are not the only inhibitory transmitters in the striatum and further experiments will be necessary to localize the respective $GABA_A$-receptors and learn more about the mechanism of their action on the release of CCK-IR.

While it is evident from our data that stimulation of $GABA_A$-receptors enhances the release of CCK-IR, it remains open whether this is the only effect mediated by these receptors. This speculation is based on the observation that isoguvacine had a bell-shaped concentration-response curve: at a rather high concentration of 5×10^{-5} M it had no effect on the release of CCK-IR, while it significantly enhanced it at a concentration of 5×10^{-6} M. Thus, high concentrations of isoguvacine may have additional, possibly artifactual effects on other striatal neurones which decrease the release of CCK-IR.

When given alone, bicuculline reduced the veratridine-induced release of CCK-IR. This effect was probably due to the antagonism of endogenous GABA. This result indicates the presence of a veratridine-induced tone of endogenous GABA at the $GABA_A$-receptors which increase the release of CCK-IR. This assumption may also explain the rather high concentrations of the agonists necessary to exert the observed effects. Whether endogenous GABA stimulates these receptors also under *in vivo* conditions has to be determined in future experiments.

In contrast to the enhancement of the release of CCK-IR observed with agonists at $GABA_A$-receptors, (−)-baclofen reduced the veratridine-induced release of CCK-IR. While (−)-baclofen is generally assumed to be an agonist selective for $GABA_B$-receptors (Bowery *et al.*, 1980; Hill & Bowery, 1981), the effects of (+)-baclofen are less clear. It binds to $GABA_B$-sites with a lower affinity than (−)-baclofen and its potency is only 1% of that of (−)-baclofen (Hill & Bowery, 1981). Because of its low intrinsic activity it has been used as an antagonist at $GABA_B$-receptors

(Schlicker *et al.*, 1984). However, its efficacy as such has been disputed (Haas *et al.*, 1985). In this study, it antagonized the effect of (−)-baclofen, but did not have a significant effect when given alone. Since a second antagonist at $GABA_B$-receptors, δ-amino-*n*-valeric acid (Muhyaddin *et al.*, 1982), also blocked the effect of (−)-baclofen on the veratridine-induced release of CCK-IR, it can be assumed that $GABA_B$-receptors are indeed involved. The concentration of (−)-baclofen necessary to elicit these effects was rather high (10^{-3} M). This is possibly due to a high concentration at the respective receptors of endogenous GABA which had been released by the stimulation with veratridine. Therefore, it is interesting to note that the concentrations of (−)-baclofen necessary to decrease the release of CCK-IR as induced by K^+ 40 mM in the presence of tetrodotoxin were considerably lower (10^{-6} M).

The observed reduction of the release of CCK-IR caused by stimulation of $GABA_B$-receptors is in agreement with previous reports that these receptors mediate the decrease of the release of several other transmitters. Thus, in the striatum, activation of $GABA_B$-receptors reduces the release of dopamine from its afferent terminals (Bowery *et al.*, 1980; Reimann *et al.*, 1982).

$GABA_B$-receptors are assumed to be located and to act presynaptically. We used GABA and (−)-baclofen in our attempts to localize the $GABA_B$-receptors which reduce the release of CCK-IR. In the presence of the uptake blocker (±)-nipecotate, GABA decreased the release of CCK-IR caused by K^+ (40 mM) in the presence of tetrodotoxin. This result indicates that the receptors which mediated the effect of GABA were either situated on the CCK-containing terminals or in their immediate vicinity. Since this effect of GABA was resistant to picrotoxin which inhibits the $GABA_A$-receptor complex (Takeuchi & Takeuchi, 1969; Ticku *et al.*, 1978), but was antagonized by δ-amino-*n*-valeric acid, which is known to block $GABA_B$-receptors (Muhyaddin *et al.*, 1982), it is probably due to stimulation of $GABA_B$-receptors. This assumption was confirmed by the finding that (−)-baclofen but not the stereoisomer (+)-baclofen decreased the release of CCK-IR release caused by K^+ (40 mM) in the presence of tetrodotoxin. This effect of (−)-baclofen was abolished by the $GABA_B$-receptor antagonist, δ-amino-*n*-valeric acid.

The importance of the $GABA_B$-receptors which decrease the release of CCK-IR has to be evaluated in further experiments. Especially, their possible role in the modulation of CCK-IR release by dopamine receptors (see Introduction) has to be clarified.

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