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Effect of quinine on autoreceptor-regulated serotonin release in the rat hippocampus

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Abstract

The involvement of K^+ channels in the autoregulation of terminal serotonin (5-hydroxytryptamine, 5-HT) release was investigated by microdialysis in the hippocampus of conscious rats. Extracellular 5-HT was increased concentration-dependently by the K^+ channel blocker quinine (10, 100 and 1000 μM in perfusate), and tetrodotoxin (10 μM) but not fluoxetine (5 μM) exerted a partially attenuating influence. The 5-$HT_{1/2/6}$ receptor antagonist methiothepin (50 μM) increased dialysate 5-HT, most likely through 5-HT_{1B} autoreceptors tonically activated in the hippocampus of *awake* rats as opposed to the previously reported lack of effect 5-HT_{1B} autoreceptor blockade in *anesthetized* rats. The effect of methiothepin was greatly reduced by preperfusion with quinine (100 μM), consonant with a role for quinine-sensitive K^+ channels in the autoregulation of 5-HT release in the hippocampus by 5-HT receptor antagonism. In contrast, the reduction in dialysate 5-HT induced by the 5-HT_1 receptor agonist RU 24969 (1 μM), in the presence of fluoxetine (5 μM), persisted in the co-presence of quinine, consonant with the involvement of (extrasynaptic?) 5-HT autoreceptors not coupled with quinine-sensitive K^+ channels. © 1997 Elsevier Science B.V.

Keywords: Hippocampus; Quinine; RU 24969; 5-HT (5-hydroxytryptamine; serotonin); Autoregulation; Microdialysis

1. Introduction

After the original finding that dopamine increases K^+ conductance in substantia nigra neurones through dopamine D_2 receptors (Lacey et al., 1987) coupled to a GTP-binding protein (Lacey et al., 1988), K^+ channels have been implicated in the D_2 autoreceptor regulation of striatal dopamine release both in vitro (Cass and Zahniser, 1990, 1991) and in vivo (Tanaka et al., 1992). More recent in vivo microdialysis studies with K^+ channel blockers or openers targeting the ATP-sensitive, inward rectifier K^+ channels indicated the likely involvement of this K^+ channel subtype (Tanaka et al., 1994, 1995, 1996). Many of the K^+ channels associated with postsynaptic dopamine D_2 receptors in patch-clamped striatal neurones were also of the inward rectifier type (Greif et al., 1995).

Although there is substantial support for the linkage of 5-HT_{1A} receptors in the 5-hydroxytryptamine (5-HT, serotonin) cell body region with K^+ channels through a GTP binding protein (Innis and Aghajanian, 1987; Innis et al., 1988; Nicoll, 1988), there is no evidence implicating K^+ channels in the autoregulation of 5-HT release in 5-HT terminal regions. With microdialysis in awake rats, 5-HT release from the striatum was found to be increased by apamin, which blocks Ca^{2+}-activated K^+ channels, and the non-selective blocker tetraethylammonium; but not by mast cell degranulating peptide, which acts at dendrotoxin-sensitive delayed-rectifier K^+ channels, or 4-aminopyridine, which has some degree of specificity for voltage-gated delayed rectifier currents and transient A currents (Dawson and Routledge, 1995). However, the latter effects may be caused by K^+ channel block-induced elongation of action potentials leading to Ca^{2+} influx which assists transmitter release (Tibbs et al., 1989) independent of autoregulation of axonal 5-HT release. In an in vitro study, Bagdy and Harsing (1995) demonstrated the involvement of 4-aminopyridine- but not ATP-sensitive K^+ channels in the regulation of somatodendritic 5-HT release by 5-HT_{1A} receptors in the raphe in agreement with earlier electrophysiological results (Haj-Dahmane et al.,

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1991); although regulation of terminal release by 5-HT_{1B} receptors in the hippocampus did occur in the former study, K^+ channel blockers were not tested in that brain region.

The present study addressed the potential involvement of K^+ channels in the autoregulation of 5-HT release in a 5-HT terminal region, the hippocampus, by microdialysis in awake rats. As a first approach towards identifying K^+ channel involvement, quinine, a blocker of ATP-sensitive, delayed rectifier, Ca^{2+}-activated, and G protein-coupled receptor-activated K^+ channels (Castle et al., 1989; Fatherazi and Cook, 1991), was used together with methiothepin, a 5-$HT_{1/2/6}$ receptor antagonist which antagonizes the action of 5-HT at 5-$HT_{1A/B/D}$ autoreceptors (Zifa and Fillion, 1992; Chopin et al., 1994), and RU 24969, a 5-HT_1 receptor agonist which activates 5-$HT_{1A/B/D}$ autoreceptors (Chopin et al., 1994; Griebel, 1995).

2. Materials and methods

2.1. Microdialysis and drug treatment

Male Sprague–Dawley rats (250–300 g; Harley Sprague–Dawley, Indianapolis, IN) were anesthetized with a combination of ketaset HCl (100 mg/kg i.p.) and halothane (5% in oxygen) for surgery. A 22-gauge guide cannula (Harvard Apparatus, Natick, MA) was stereotaxically implanted over the hippocampus without penetrating the dura. The coordinates relative to bregma were: anteroposterior 5.2 mm, L 4.8 mm (Paxinos and Watson, 1986). All animal procedures adhered strictly to institutional and national ethical guidelines. On the test day, 5 d after guide implantation, a probe was inserted, constructed from cellulose acetate dialysis fibers (I.D. 215 ± 15 microns, molecular weight cutoff = 6000; Spectrum Medical Industries, Los Angeles, CA) attached to fused silica tubing (Polymicro Technologies) with a loop-type dialysis area of 6 mm dialyzing a 3 mm deep brain area. The tip of the dialysis probe was planned to be 7.2 mm below the dura. The probes were perfused in freely moving animals at a flow rate of 1.6 μl per minute with artificial cerebrospinal fluid containing 150 mM Na^+, 3 mM K^+, 1.2 mM Ca^{2+}, 0.8 mM Mg^{2+}, 155 mM Cl^- for 3–4 h until dialysate 5-HT levels had stabilized. After collecting three baseline dialysate samples, infusion of a compound started as indicated in the figures, followed as needed by co-infusion of a second compound. When fluoxetine was present, it was infused continuously starting after insertion of the dialysis probe. Drug delivery and sample collection time were corrected for the lag time resulting from the dead volume of the inlet and outlet tubes. In vitro probe recovery for 5-HT was always determined and varied between 10 and 15%. The absolute levels reported in this work for dialysates under basal conditions were not corrected for probe recovery.

2.2. HPLC and histology

For serotonin determination, dialysates were analyzed immediately following collection by HPLC as described previously (Yan et al., 1992) with an HR-80 column (ESA, Chelmsford, MA) and a mobile phase of 75 mM NaH_2PO_4, 1.5 mM sodium dodecyl sulfate, 20 μM EDTA, 100 μl/l triethylamine (pH 5.6 with H_3PO_4), 12% methanol, and 12% acetonitrile, pumped at 1 ml/min by a model 580 ESA pump. Detection was of the electrochemical type (ESA, Coulochem II) equipped with a dual electrode analysis cell (M 5014) and a guard cell (M 5020). The guard cell was set at 400 mV, electrode 1 at −100 mV, and electrode 2 at 225 mV with respect to palladium reference electrodes. The elution time for 5-HT was approximately 11 min, and the detection limit was 1 fmol at a 2:1 signal to noise ratio. Integration of peaks from chromatograms was carried out with an EZChrom chromatographic software system (Scientific Software, San Ramon, CA).

Placements of the probes were verified in coronal sections stained with cresyl violet as described previously (Chen and Reith, 1994a; Li et al., 1996).

2.3. Drugs

Fluoxetine and desipramine were gifts from Lilly Research Laboratories (Indianapolis, IN) and USV Laboratories (Tuckahoe, NY) respectively. Methiothepin maleate was a gift from Hoffman–La Roche, (Nutley, NJ) and RU 24969 was purchased from Research Biochemicals (Natick, MA) All other chemicals, including tetrodotoxin (TTX), were from Sigma (St. Louis, MO). Compounds were freshly prepared by dissolving in artificial cerebrospinal fluid; stocks were prepared at 1 mM and further dilutions were made in artificial cerebrospinal fluid for the desired concentrations.

2.4. Data analysis

Levels of dialysate 5-HT are reported as percentage of baseline control measures, i.e. the average of the three samples immediately preceding drug administration. Comparisons of 5-HT time courses between experimental conditions were carried out by a two-way ANOVA with repeated measures over time (factor A = condition, factor B = time); for the comparison of drug effects after pretreatment with TTX or fluoxetine, the response *over* the newly established pretreatment level was analyzed along with the response *over* baseline for the group without pretreatment. Individual time-point values between no more than two groups were compared with the unpaired Student's *t*-test. Changes in 5-HT values compared with the pretreatment baseline values were assessed with the one-sample Student's *t*-test. The accepted level of significance was 0.05, two-tailed.

3. Results

3.1. Quinine in the absence or presence of TTX or fluoxetine

Quinine increased hippocampal 5-HT dialysate levels concentration-dependently (Fig. 1A). Upon infusing 10 and 100 μM quinine for 80 min, 5-HT levels appeared to reach a plateau, whereas the highest concentration tested, 1000 μM, caused a dramatic, but transient 5-HT increase. TTX (10 μM) substantially reduced dialysate 5-HT to approximately 35% of baseline, and attenuated but did not prevent the 5-HT increase induced by 1000 μM quinine (Fig. 1B). Fluoxetine (5 μM) by itself increased the absolute 5-HT baseline levels in hippocampal dialysates from 11 ± 1 fmol/40 μl (mean $\pm$ S.E. for 28 dialyzed animals in the absence of fluoxetine) to 30 ± 2 fmol/40 μl (ibid with $n = 28$ in the presence of fluoxetine) ($P < 0.0001$, Student's t-test). Compared with the newly established level of 5-HT in the dialysate, quinine (100 μM) was as effective in increasing 5-HT in the presence of fluoxetine (Fig. 1C) as in its absence (Fig. 1A).

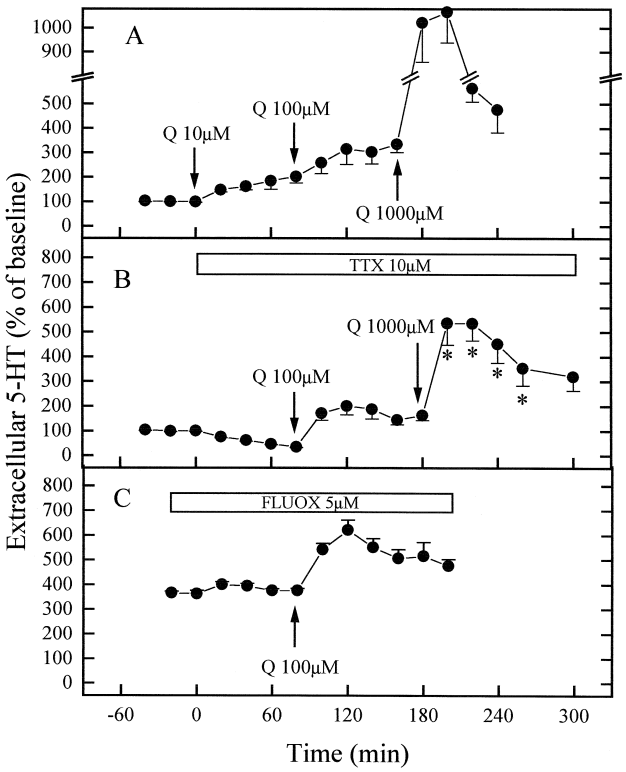

Fig. 1. Effects of local infusion of quinine (Q) into the hippocampus on the levels of hippocampus dialysate 5-HT in the absence (A) or presence of tetrodotoxin (TTX) (B) or fluoxetine (FLUOX). Horizontal boxes denote the period of infusion with compound as indicated inside the box. The arrows indicate the first sample after infusion of each concentration of Q. Values are mean $\pm$ S.E. for 7–8 animals. All points at less than 75% or more than 125% of baseline were statistically significant ($P < 0.05$, one-sample Student's t-test compared with 100%). $^{*}P < 0.05$ ($F(1,14) = 5.84$), compared with 1000 μM Q alone (two-way ANOVA with repeated measures on responses over pre-quinine baseline; listed F-value is for condition factor, see Section 2).

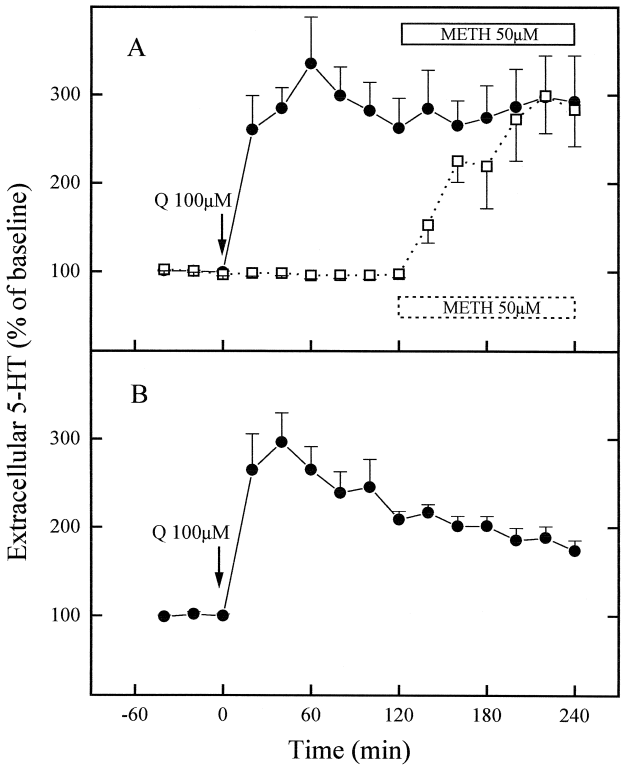

Fig. 2. Effects of local infusion of quinine (Q) into the hippocampus on the levels of hippocampus dialysate 5-HT in the presence of methiothepin (METH) (A) or in its absence (B) and the effect of METH by itself (A). Horizontal boxes with each curve denote the period of infusion with compound as indicated inside the box. The arrows indicate the first sample after infusion of Q. Values are mean $\pm$ S.E. for 6–7 animals. All points at more than 125% of baseline were statistically significant ($P < 0.05$, one-sample Student's t-test compared with 100%). Two-way ANOVA with repeated measures did not show a statistically significant difference between the Q curves with and without METH ($P = 0.11$ with $F(1,12) = 2.91$ for condition factor and $P = 0.07$ with $F(14,154) = 1.64$ for condition $\times$ time factor, see Section 2).

3.2. Methiothepin in the absence or presence of quinine

Methiothepin (50 μM) by itself appreciably increased dialysate 5-HT (dotted curve in Fig. 2A). Infusion of quinine (100 μM) for 2 h increased 5-HT, as in the experiments described above, and the subsequent co-presence of methiothepin (50 μM) did not enhance 5-HT above % baseline levels seen with methiothepin alone (solid curve in Fig. 2A). When quinine (100 μM) alone was tested on the same time schedule (Fig. 2B), the curve was similar to that for quinine followed by co-addition of methiothepin (Fig. 2A); the third and fourth hour portion of the latter appeared to describe relatively higher % baseline values, suggesting a small additional effect of the co-presence of methiothepin, but this effect did not reach statistical significance ($P = 0.073$ with $F(14,154) = 1.64$ for condition $\times$ time in two-way ANOVA with repeated measures over time).

3.3. Quinine prior to RU 24969 or vice versa

In the following experiments, fluoxetine (5 μM) was infused continuously in order to be able to study the 5-HT receptor agonist effect of RU 24969 (1 μM). This was necessary because of the serotonin transporter blocking effect of RU 24969 which causes an increase in dialysate 5-HT obliterating the reducing effect on 5-HT release through 5-HT autoreceptor activation (Auerbach et al., 1990; Hjorth and Tao, 1991; Martin et al., 1992; Bosker et al., 1995). Preliminary experiments without fluoxetine confirmed the previously reported observations (data not shown). In the presence of fluoxetine, RU 24969 reduced 5-HT in hippocampal dialysate, and the subsequent co-presence of quinine (100 μM) caused an appreciable increase towards % baseline values observed generated by quinine alone (Fig. 3A). The co-presence of RU 24969 appeared to have a small additional effect as indicated by the lower % baseline levels of 5-HT in the presence than absence of RU 24969 in the last 40 min of the 2 h quinine infusion (solid versus dotted curve in Fig. 3A). This was more clear when the order of addition was reversed (Fig. 3B). Infusion of RU 24969 (1 μM) during the last 2 h of a 4 h quinine (100 μM) application caused % baseline values of 5-HT to drop below those seen with quinine alone ($P < 0.05$, Student's *t*-tests following a significant condition × time factor ($F(14,168) = 1.97$, $P < 0.05$) in two-way ANOVA with repeated measures over time).

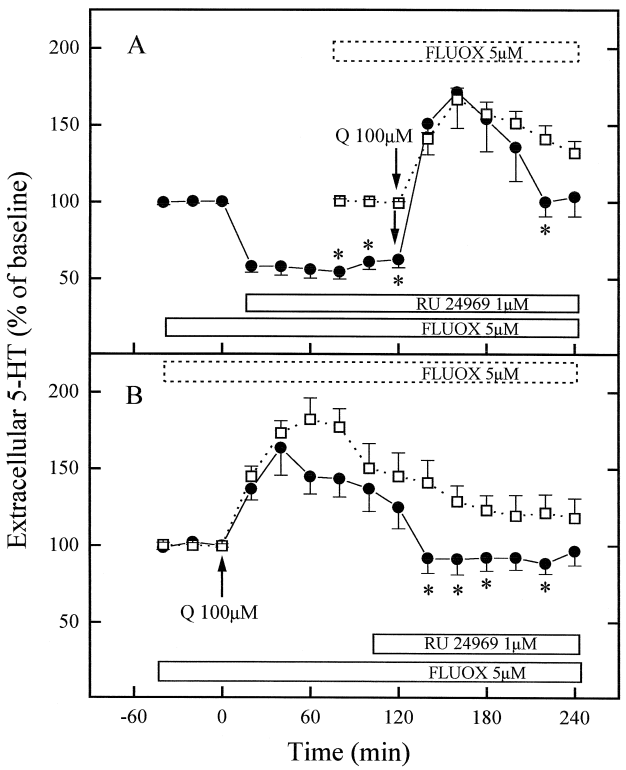

Fig. 3. Effects of local infusion of quinine (Q) into the hippocampus on the levels of hippocampus dialysate 5-HT in the presence of fluoxetine (FLUOX), either after (A) or prior to (B) infusion of RU 24969. Horizontal boxes with each curve denote the period of infusion with compound as indicated inside the box. The arrows indicate the first sample after infusion of Q. Values are mean ± S.E. for 7–21 (panel A) or 7 animals (panel B). All points at less than 75% or more than 125% of baseline were statistically significant ($P < 0.05$, one-sample Student's *t*-test compared with 100%). * $P < 0.05$ compared with corresponding control value at that time point (Student's *t*-test). In panel B, the two entire curves were compared with two-way ANOVA with repeated measures ($F(14,168) = 1.97$, $P < 0.05$ for condition × time factor, see Section 2).

4. Discussion

4.1. 5-HT_{1B} receptors and methiothepin-induced 5-HT increase

Although methiothepin is a non-selective 5-$HT_{1A,B,D,2,6}$ receptor antagonist (Zifa and Fillion, 1992; Humphrey et al., 1993; Chopin et al., 1994), autoreceptors controlling the release of 5-HT in 5-HT terminal regions have been shown to be mostly of the 5-HT_{1B} subtype in rodents and of the 5-HT_{1D} subtype in other species including man (for references see Chopin et al., 1994). Therefore, it is reasonable to consider the increase in extracellular 5-HT observed following methiothepin infusion into the hippocampus as mediated by 5-HT_{1B} receptors. These receptors, then, must be tonically activated to some extent by endogenous 5-HT for the antagonist to be effective by itself. This stands in contrast to observations reported by Hjorth and Sharp (1993) who showed that infusion of the 5-$HT_{1A,B}$ antagonist (−)-penbutolol into the hippocampus enhanced dialysate 5-HT only when the 5-HT uptake blocker citalopram was present in the perfusion medium, indicating the need to elevate endogenous 5-HT tone for uncovering the antagonist effect. In contrast, when citalopram was given systemically, intrahippocampal (−)-penbutolol had no effect presumably because of reduced 5-HT cell firing due to increased 5-HT at somatodendritic 5-HT_{1A} receptors (Hjorth, 1993). The discrepancy between the present finding of a 5-HT increase following intrahippocampal methiothepin and the lack of effect of (−)-penbutolol in the experiments of Hjorth and Sharp (1993), in the absence of local 5-HT uptake blockade, is likely caused by the use of anesthetized animals in the latter studies. A general anesthetic can have a profound effect in reducing the response of serotonergic raphe neurons to auditory stimulation (Heym et al., 1982) and drug treatment (Trulson and Trulson, 1983) presumably by enhanced GABA release depressing 5-HT activity (Soubrie et al., 1983). Thus, the level of normal arousal in awake rats undergoing dialysis is likely to increase 5-HT in 5-HT terminal regions, resulting in tonic activation of 5-HT_{1B} autoreceptors. In fact, we found local infusion of methiothepin through a probe in the ventral tegmental area to be effective in enhancing dialysate 5-HT in awake rats in the absence of local serotonin uptake blockade (Chen and Reith, 1994b). Fur-

thermore, Renyi et al. (1991) reported that systemic administration to rats of isamoltane, a β-adrenoceptor antagonist with appreciable 5-HT_{1B} and much less 5-HT_{1A} blocking activity, induced wet-dog shakes preventable by the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine. This behavior was also blocked by ritanserin, suggesting mediation by the serotonergic system. The data taken together suggest that in awake animals, at least in some brain regions, terminal 5-HT_{1B} receptors are tonically activated by endogenous 5-HT.

The possibility could be considered that methiothepin which has a an affinity for 5-HT_{1A} receptors only slightly lower than for 5-HT_{1B} receptors (Zifa and Fillion, 1992; Chopin et al., 1994; Griebel, 1995) interferes with the action of 5-HT in the hippocampus on a large postsynaptic 5-HT_{1A} receptor-mediated feedback loop speculated to be present from experiments with the 5-HT_{1A} agonist 8-OH-DPAT (Blier and de Montigny, 1987; Ceci et al., 1994; Romero et al., 1994). However, no information is available on effects of 5-HT_{1A} antagonists that might be mediated by this circuit; such antagonists would be expected to be ineffective via the postulated fronto–cortical pathway, removal of which does not modify firing of raphe 5-HT cells (Ceci et al., 1994). More recent evidence of Jolas et al. (1995) argues against the existence of a feedback loop originating in the hippocampus (for a discussion see De Vry, 1995).

4.2. Nature of quinine-induced 5-HT increase

The neuronal resting membrane potential, negative inside, is less than the equilibrium potential for K^+ as a result of the slight permeability of the nerve membrane to Na^+ (Koester, 1996). This is likely to be true for nerve endings as well as cell bodies. Thus, brain synaptosomal preparations have estimated resting potentials close to those measured in mammalian central neurons (Blaustein and Goldring, 1975), and there were no differences in resting membrane potentials measured at the soma, neurite varicosity and growth cone of rat dorsal ganglion neurons in culture (Wang et al., 1994). Thus, in analogy to the case of DA autoreceptors in the striatum (Cass and Zahniser, 1990, 1991; Tanaka et al., 1992, 1994), if occupation of 5-HT autoreceptors by endogenous 5-HT were to open K^+ channels, it would be expected to hyperpolarize the membrane, inhibiting transmitter release. If this were true for 5-HT in the hippocampus, blockade of K^+ channels could be thought to counteract this effect of endogenous 5-HT, resulting in increased 5-HT release. Therefore, the non-selective K^+ channel blocker quinine could increase dialysate 5-HT through this mechanism. In addition, K^+ channels not linked to autoreceptors could play a role; blockade of such channels by quinine would be expected to assist membrane depolarizations associated with neuronal impulse flow. However, other processes must play an important role, because TTX, which reduces neuronal impulse-dependent 5-HT release by blocking voltage-dependent Na^+ channels (see Chen and Reith, 1994a), only partially diminished the effect of quinine (1000 μM). Quinine's action in enhancing dialysate dopamine in the striatum has been proposed to involve dopamine efflux through the dopamine transporter operating in reversed mode (Tanaka et al., 1992), but in the present experiments fluoxetine, a 5-HT uptake blocker, did not attenuate the effect of quinine (100 μM) on dialysate 5-HT. It is possible that quinine, by blocking K^+ channels carrying the resting membrane potential such as Ca^{2+}-activated maxi-K and SK channels (Nicholls et al., 1992), causes depolarization which in turn activates Ca^{2+} influx and transmitter release. Indeed, a quinine (0.2 mM) induced depolarization of the resting membrane potential of 14 mV has been reported for rat dorsal root ganglion neurons in culture (Wang et al., 1994).

4.3. 5-HT autoreceptor (ant)agonism and K^+ channels

The present observation that the effect of methiothepin in enhancing dialysate 5-HT is largely lost when quinine is co-infused, argues in favor of the involvement of quinine-sensitive K^+ channels in the antagonism of 5-HT autoreceptor regulation. Thus, when putative autoreceptor-associated K^+ channels are blocked with quinine, methiothepin cannot antagonize the action of endogenous 5-HT in opening these channels, i.e. preblocking the K^+ channels with quinine renders the autoreceptor insensitive to the action of either agonist (5-HT) or antagonist (methiothepin). Of course, the present evidence for 5-HT_{1B} autoreceptor associated K^+ channels is preliminary, and other K^+ channel blockers and 5-HT_{1B} autoreceptor agents need to be tested. Although no electrophysiological evidence exists for the coupling of this 5-HT receptor subtype to K^+ channels, as opposed to the large body of evidence for the 5-HT_{1A} subtype (Innis and Aghajanian, 1987; Innis et al., 1988; Nicoll, 1988), this should not be taken as evidence against such a coupling for the 1B subtype, because its location on nerve terminals does not make it accessible for in situ electrophysiological techniques. Presynaptic 5-HT autoreceptors are believed to differ from somatodendritic autoreceptors in that they do *not* alter impulse transmission but rather alter some aspect of stimulus–secretion coupling such as influx of Ca^{2+} though voltage-dependent Ca^{2+} channels (Feldman et al., 1997). This is based on a single study in which Göthert (1980) made the interesting observation that Ca^{2+}-induced [^{3}H]5-HT release from already depolarized cerebrocortical slices was enhanced by methiothepin in the same concentration range as K^+-induced release. However, more information is needed on the in vitro Ca^{2+}-induced [^{3}H]5-HT release as a paradigm.

It could be argued that quinine interferes with the action of methiothepin in a nonspecific manner, via depolariza-

tion (see above). In this line of reasoning, the quinine-induced membrane depolarization, perhaps involving voltage-dependent Ca^{2+} channels, stimulates 5-HT release in a ceiling effect, which prevents methiothepin from further stimulating 5-HT release. Under non-depolarized conditions, methiothepin could be thought to remove agonist-induced inhibition of such Ca^{2+} channels. However, this line of thought is opposed by the observation that K^+-induced depolarization-evoked 5-HT release is in fact enhanced by methiothepin in slices of 5-HT terminal areas such as frontal cortex (Middlemiss, 1984), hypothalamus (Passarelli et al., 1987), and hippocampus (Passarelli et al., 1988).

In contrast to the interaction of quinine with the 5-HT receptor antagonist methiothepin, activation of 5-HT receptors by the agonist RU 24969 still caused a decrease in hippocampal dialysate 5-HT in the presence of quinine. This is reminiscent of the ability of quinine to block the increase in striatal extracellular DA induced by the DA receptor antagonist sulpiride but not the decrease following the agonist quinpirole or N-0437 (Tanaka et al., 1992). In analogy to the suggestion made by Tanaka et al. (1992), two populations of 5-HT autoreceptors could be postulated. First, there are synaptic autoreceptors, tonically activated by endogenous 5-HT, and blockable by an antagonist such as methiothepin which increases 5-HT release; this involves quinine-sensitive K^+ channels. Exogenously applied 5-HT receptor agonists would have little or no effect because of the tonic activation by endogenous 5-HT. Second, there may be extrasynaptic 5-HT autoreceptors not tonically activated by 5-HT that can be acted upon by exogenously added agonist, causing a reduction in 5-HT release, but these release-regulating autoreceptors are not coupled with quinine-sensitive K^+ channels. There is substantial experimental support for the existence of extrasynaptic receptors for monoamines including 5-HT (Carlsson et al., 1969; Descarries et al., 1991), sometimes referred to as volume transmission (for references see Agnati et al., 1995).

Passarelli et al. (1988) reported that pertussis toxin abolished the inhibitory effect of 5-HT autoreceptor activation on 5-HT release from hippocampal slices, but more recent work of Blier suggest that release-regulating autoreceptors on serotonergic terminals in the hippocampus are not coupled to G_i, G_s, or G_o proteins (Blier, 1991). It is possible that the transduction mechanism relating terminal 5-HT autoreceptors to K^+ channel gating is different from the G-protein coupled type involving ATP-sensitive K^+ channels advanced for the action of dopamine autoreceptor antagonists (Tanaka et al., 1995, 1996). Clearly, K^+ channels can be modulated by many indirectly coupled receptors, involving second messengers such as cAMP, diacylglycerol, and arachidonic acid (Nicholls et al., 1992), and more work is needed to delineate the channel subtype, and coupling, involved in terminal 5-HT autoregulation.

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