The 5-HT₄ receptor subtype inhibits K⁺ current in colliculi neurones via activation of a cyclic AMP-dependent protein kinase

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1 The aim of the present study was to examine the effect of 5-hydroxytryptamine (5-HT) on K⁺ current in primary culture of mouse colliculi neurones and to identify the 5-HT receptor subtype that could be involved in this effect.

2 The voltage-activated K⁺ current of the neurones was partially blocked by 8-bromo adenosine 3':5'-cyclic monophosphate (8-bromo-cyclic AMP). This effect was mimicked by 5-HT and the action of 5-HT could be antagonized by H7, a non specific protein kinase inhibitor, and by PKI, the specific cyclic AMP-dependent protein kinase blocker.

3 A similar cyclic AMP-dependent blockade of the K⁺ current was found with renzapride (BRL 24 924) and other 5-HT₄ receptor agonists such as cisapride, BIMU 8, zacopride and 5-methoxytryptamine (5-MeOT). ICS 205 930, the classical 5-HT₄ receptor blocker, could not be used in this study because it inhibited the studied K⁺ current by itself. However, the novel 5-HT₄ receptor antagonist, DAU 6285 blocked the effects of 5-HT and renzapride on the K⁺ current.

4 The current was insensitive to the 5-HT₁ and 5-HT₃ receptor agonists (8-hydroxy-2-(di-n-propyl-amino) tetralin, RU 24 969, carboxamidotryptamine, 2-CH₃-5-HT) as well as to 5-HT₁, 5-HT₂ and 5-HT₃ antagonists (methiothepin, ketanserin, ondansetron [GR 38 032]). Moreover, these antagonists did not affect the actions of the tested 5-HT₄ receptor agonists.

5 The present results show that part of the voltage-activated K⁺ current in mouse colliculi neurones is cyclic AMP-sensitive and the blockade of the current by 5-HT involves the 5-HT₄ receptor subtype. The putative implication of 5-HT₄ receptors in neuronal plasticity, via a blockade of K⁺ channels, is discussed.

Keywords: 5-Hydroxytryptamine; 5-HT₄ receptor; K⁺ current; 'patch-clamp'; neuronal plasticity

Introduction

A variety of mechanisms have been described to trigger modulation of K⁺ channels by neurotransmitters. Phosphorylation/dephosphorylation of the channel itself or some modulatory intermediate events are the most common mechanisms (Rudy, 1988; Brown, 1990). Phosphorylation is the result of protein kinase C (PKC; Akerman *et al.*, 1988; Doerner *et al.*, 1988), adenosine 3':5'-cyclic monophosphate (cyclic AMP)-dependent protein kinase (PKA; Siegelbaum *et al.*, 1982; Deterre *et al.*, 1982; Ewald *et al.*, 1985) or other Ca²⁺-activated protein kinase or phosphatase (Rudy, 1988; Hoyer *et al.*, 1991). Other mechanisms have been described, including direct interaction of Gi/Go proteins (Yatani *et al.*, 1987; Von Dongen *et al.*, 1988) or arachidonic acid metabolites (Piomelli *et al.*, 1987).

The physiological roles of such K⁺ channel regulations by second messengers and transmitters are not always known. However, in *Aplysia californica* a cellular biological approach has demonstrated that short-term and long-term sensitizations of the gill withdrawal reflex is due in part to a 5-HT receptor-activated cyclic AMP cascade (Kandel & Schwartz, 1982; Greenberg *et al.*, 1987). 5-HT receptors localized on sensory neurones increase cyclic AMP production and therefore PKA activation, leading to closure of K⁺ channels, prolonged depolarization, opening of Ca²⁺ channels and then enhanced transmitter release. These 5-HT-inhibited K⁺ channels in *Aplysia californica* neurones are specific K⁺ channels called S-K⁺ channels. They are active at resting membrane

potential, do not inactivate and do not participate in the fast (A-current), delayed and Ca²⁺-activated K⁺ currents (Klein *et al.*, 1982). This model of presynaptic facilitation is quite interesting because it may represent a cellular model of short- or long-term synaptic plasticity. However, such a model has not yet been described in vertebrates.

Recently, we have found in colliculi and hippocampal neurones a novel 5-HT receptor subtype having a pharmacology that differs strictly from those of the already described 5-HT₁, 5-HT₂ and 5-HT₃ receptors. We designated this new receptor as the 5-HT₄ receptor (Dumuis *et al.*, 1988, 1989; Bockaert *et al.*, 1990). It stimulates cyclic AMP production and has now been described in guinea-pig ileum and colon, rat oesophagus and human heart (Craig & Clarke, 1990; Craig *et al.*, 1990; Kaumann *et al.*, 1990; Ouadid *et al.*, 1991). In guinea-pig ileum, 5-HT₄ receptors are likely localized on myenteric cholinergic neurones and trigger acetylcholine release (Pfeuffer-Friederich & Kilbinder, 1984; Buchheit *et al.*, 1985).

It is possible that 5-HT₄ receptors in vertebrates have similar physiological roles in neuronal plasticity as other 5-HT receptors do in *Aplysia californica*. In order to investigate this question, the first step was to analyze the possibility that 5-HT₄ receptors trigger a cyclic AMP-mediated inhibition of K⁺ current in colliculi neurones.

Methods

Colliculi were dissected from 14–15 days old mouse embryos. Cultures were prepared as previously described for striatal

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neurones (Weiss *et al.*, 1986). Briefly, neurones were dissociated and plated in 35 mm diameter dishes containing a defined culture medium supplemented with 2% serum and hormone mixture. They were maintained at 37°C in a humidified atmosphere and in the presence of 5% CO₂/95% O₂ for 5–10 days before electrophysiological recording.

Patch-clamp experiments were performed at room temperature. The culture medium was replaced by a solution containing (mM): Cl⁻ 139, K⁺ 5, Na⁺ 130, Ca²⁺ 2, Mg²⁺ 2, HEPES 10 and tetrodotoxin 0.3 µM (pH 7.4). The whole-cell recording pipettes were filled with a solution of the following composition (mM): Cl⁻ 137, K⁺ 130, Na⁺ 8.5, Mg²⁺ 2, Ca²⁺ 0.5, EGTA 5, HEPES 10, ATP 4 and GTP 0.5 (pH 7.2). Under these conditions, the impedances of our recording electrodes were between 3 and 5 MΩ. Voltage-activated K⁺ currents were recorded using a LIST EPC7 amplifier and digitized at 1 kHz for storage and analysis on a PC compatible computer. They were digitized at 100 Hz for iconography. Linear leak and capacitive currents were subtracted. All these computerized procedures were performed with the pClamp 5.5.1 programme of Axon Instruments.

Drug solutions were prepared in these media (either external or internal medium, as indicated in the text and figure legends) and pH of the solutions was adjusted at the appropriated value (see here above). External drug applications were performed by use of an 8 barreled fast perfusion system. One mM isobutylmethylxanthine (IBMX) and 0.1 µM forskolin were applied before (control conditions) and during perfusion of all the tested 5-HT receptor agonists. Pargyline (10 µM) and ascorbic acid (1%) were added to the external solutions in order to prevent oxidative degradation of 5-HT. At the concentrations used, neither IBMX nor forskolin nor the antioxidative agents, applied alone or together, modified the studied K⁺ current, in the absence of agonist. These observations corroborate our previous studies showing that 0.1 µM forskolin, in the presence of 1 mM IBMX, does not modify basal cyclic AMP concentrations but increases neurotransmitter efficacy in cyclic AMP production, whereas potency remains unaffected (see Figure 1 in article by Weiss *et al.*, 1985).

The following drugs were purchased from SIGMA: ATP-Na₂, GTP-Na, 5-HT, the protein kinase inhibitor peptide (PKI), 8-bromo-cyclic-AMP, H7 (1-[5-isoquinolinesulfonyl]-2-methylpiperazine), tetrodotoxin, IBMX, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) forskolin and 5-MeOT. The following drugs were generously donated: ICS 205 930 ([3-α-tropanyl]-1H-indole-3-carboxylic acid ester and 2-CH₃-5-HT; (Sandoz, Basel, Switzerland), methiothepin (Hoffmann-Laroche, Basel, Switzerland), cisapride ([*cis*]-4-amino-5-chloro-N-[1-[3-(4-fluorophenoxy) propyl]-3-methoxy-4-piperidinyl]-2-methoxy-benzamide) and ketanserin (Janssen

Figure 1 Cyclic AMP-sensitivity of the voltage-activated K⁺ current. In this and all the following figures, the holding potential was -60 mV and depolarizing steps to +30 mV (lower trace in a) served to evoke the recorded outward currents. In (a and b) trace 1 represents the control recordings and trace 2 is the current obtained in the presence of 100 µM external 8-bromo-cyclic AMP in (a) and in the presence of 5 µM external forskolin in (b). (a) and (b) are from two different cells. Similar results were obtained in 11 other cells recorded in the presence of both drugs.

Pharmaceutica, Beerse, Belgium), 5-carboxamidotryptamine (5-CT) and ondansetron (or GR 38 032; Glaxo Group Research, Hertfordshire, UK), RU 24 969 (Roussel-Uclaf, Romainville, France), zacopride (4-amino-N-[1-azabicyclo[2.2.2]oct-3-yl]-5-chloro-2-methoxy-benzamide HCl; Delalande, Paris, France), BIMU 8 ((endo-N-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-iso-propyl-2-oxo-1H-benzimidazol-1-carboxamide HCl) and DAU 6285 (endo-6-methoxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylate hydrochloride quinuclidinyl benzilate Boehringer Ingelheim, Italy), renzapride (or BRL 24 924; ([±]-endo)-2-methoxy-4-amino-5-chloro-N-(1-azabicyclo[3.1.1]non-4-yl)-benzamide HCl; Beecham Pharmaceutica, Harlow, U.K.).

Results

Inhibition of a K⁺ current in colliculi neurones by cyclic AMP

We examined membrane currents of cultured colliculi neurones using the whole-cell configuration of the patch-clamp technique. Membrane potential was clamped at -60 mV and outward currents were evoked by depolarizing steps of various amplitude. The current-voltage relationship shifted to the right with increasing external K⁺ concentration ([K⁺]_o). Under symmetrical K⁺ concentration ([K⁺]_o/[K⁺]_i = 130 mM/130 mM) the current reversed at 0 mV, which is the theoretical equilibrium potential for K⁺ ions, showing that the outward current studied was mainly carried by K⁺ ions. This conclusion was further strengthened by the fact that the current was completely blocked by replacing internal K⁺ ions (130 mM) by Cs⁺ ions in the whole-cell recording pipette solution. It was also sensitive to tetraethylammonium ions and to 4-aminopyridine (data not shown).

The membrane-permeant analogue of cyclic AMP, 8-bromo-cyclic AMP, applied outside the cell, decreased the recorded current (Figure 1a). At concentrations of 50 and 100 µM, the decreases were of 13 ± 3% and 40 ± 8% (mean ± s.d., n = 6), respectively. Forskolin, an activator of adenylate

Figure 2 Cyclic AMP-dependent protein kinase (PKA) was involved in the 5-hydroxytryptamine (5-HT)-induced inhibition of the K⁺ current. Currents were evoked according to the procedure mentioned in Figure 1. In (a) and (c) trace 1 represents the control current obtained in two different cells; trace 2 in (a) represents the current recorded in the presence of 1 µM external 5-HT; trace 2 in (c) was obtained in the presence of 5 µM external 5-HT and 1 µM protein kinase inhibitor (PKI) in the internal solution. (b) Current-voltage relations of the outward membrane currents represented in (a) in the absence (●) and in the presence (○) of 1 µM 5-HT. Similar effects were found in 10 other cells. (d) 5-HT dose-response curves obtained from 16 cells; 8 cells recorded with normal pipette solution (●) and 8 other cells recorded with a pipette solution containing 1 µM PKI (○). Each point represents mean with s.e.mean shown by vertical bars.

cyclase, mimicked the effect of 8-bromo-cyclic AMP on the K⁺ current (Figure 1b). At 0.5 μ M, the drug inhibited the K⁺ current by $15 \pm 3\%$. Maximal effect was found at 5 μ M where forskolin induced $43 \pm 9\%$ inhibition. External IBMX (1 mM) did not affect the action of 8-bromo-cyclic AMP but doubled the effect of threshold concentration (0.5 μ M) of forskolin (data not shown). In the light of these results, it is likely that at least 40% of the outward K⁺ current in cultured collicular neurones was regulated by cyclic AMP.

Inhibition by 5-HT of the K⁺ current of colliculi neurones via a cyclic AMP-dependent protein kinase

5-HT also decreased the K⁺ current up to 30% (Figure 2a and b). The threshold concentration for the 5-HT effect was 1 nM and the maximal 5-HT effect (30% decrease) was obtained at an agonist concentration of 0.1 μ M. Half-maximal decrease of the K⁺ current was observed with concentrations of 5-HT close to 10 nM (Figure 2d, open circles).

PKI, a specific inhibitor of PKA, was perfused into the cell via the recording pipette at a concentration of 1 μ M. Basal voltage-activated K⁺ currents were measured in 10 cells dialyzed with PKI and compared to currents recorded in 11 other cells from the same culture that had been dialyzed with normal recording pipette solution. We found mean values ($\pm$ s.e.mean) of 490 ($\pm$ 48) and 350 ($\pm$ 64) pA, respectively and these values were not significantly different from each other (Student's *t*-test, $P \leq 0.05$). We then examined the effect of PKI on the 5-HT response in the same groups of cells. As shown in Figure 2c and d, PKI reduced the 5-HT-induced inhibition of K⁺ current. The PKI effect was higher at low 5-HT concentrations (Figure 2d). Similarly, the non-specific protein kinase inhibitor, H7 (0.1 μ M), completely blocked the 5-HT effect without significantly affecting the basal K⁺ current in the absence of agonist (data not shown).

The phosphatase inhibitor, okadaic acid (10 nM), alone did not alter the K⁺ current. However, in the same cells ($n = 5$) the drug markedly potentiated the effects of low concentrations of renzapride (10⁻⁹ M) and 5-HT (5 $\times$ 10⁻⁹ M). Examples of these effects are illustrated in Figure 3.

The 5-HT receptor involved in inhibition of K⁺ current in colliculi neurones is not a 5-HT₁, 5-HT₂ or 5-HT₃ receptor subtype

Several typical 5-HT receptor antagonists of the classical 5-HT receptor subtypes (5-HT₁, 5-HT₂ and 5-HT₃) were

Figure 3 The renzapride and 5-hydroxytryptamine (5-HT)-induced inhibition of the K⁺ current involved phosphorylation processes. Currents were evoked as in Figure 1. In (a and b) trace 1 is the control current and trace 2 is obtained in the presence of renzapride (10⁻⁹ M) in (a) and 5-HT (5 $\times$ 10⁻⁹ M) in (b). Trace 3 is recorded in the presence of okadaic acid (10 nM) and renzapride in (a) or 5-HT in (b) (same concentrations as above). Okadaic acid alone did not alter the control currents. Traces in (a) and (b) were obtained from the same cell.

tested on 5-HT responses equal to approximately 50% of the maximal response. Methiothepin, a 5-HT_{1A} receptor antagonist, ketanserin, a 5-HT₂ receptor antagonist and ondansetron, a 5-HT₃ receptor antagonist applied separately at a concentration of 5 μ M were unable to affect the response induced by 10 nM 5-HT (data not shown). Similarly, these compounds applied altogether did not affect either the basal K⁺ current or the inhibition of the current induced by 5-HT (10 nM; Figure 4a).

Conversely, 5-HT₁ and 5-HT₃ specific receptor agonists were tested on the K⁺ current at concentrations (see legend of Figure 4b–e) that should have fully occupied these receptors. 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and RU 24 969, two 5-HT_{1A} receptor agonists, 5-CT which activates 5-HT₁ receptor types and 2-CH₃-5-HT, a 5-HT₃ receptor agonist were all inactive on this K⁺ current (Figure 4b–e).

The receptor that inhibits K⁺ current in colliculi neurones is of the 5-HT₄ receptor subtype

Since these results suggested that none of the most classical receptor subtypes were involved in the observed 5-HT-induced inhibition of the K⁺ current, we examined the possibility that 5-HT mediated its effect via the newly discovered 5-HT₄

Figure 4 Absence of 5-HT₁-, 5-HT₂- and 5-HT₃-mediated effects on K⁺ current. Currents were evoked (see legend Figure 1) in 5 different cells. (a) Trace 1 represents the control current. Trace 2 (a) is obtained in the presence of a mixture of 5-hydroxytryptamine (5-HT) receptor antagonists (see text); methiothepin, ketanserin and ondansetron (all at 5 μ M). Trace 3 (a) is recorded in the presence of 5-HT (10 nM) alone and trace 4 (a) in the presence of 5-HT (same concentration) in the presence of the mixture of antagonists with which trace 2 was obtained. Note the absence of effect of the 5-HT receptor antagonist mixture on the currents recorded in the absence of 5-HT and the absence of effect of these antagonists on the action of 5-HT. Similar results were obtained in 5 other cells. (b–e) Trace 1 represents control current. Trace 2 is obtained in the presence of 8-hydroxy-2-(di-n-propylamino)tetralin (1 μ M; b), RU 24 969 (1 μ M; c), 2-CH₃-5-HT (10 μ M; d) and 5-carboxamidotryptamine (1 μ M; e). Similar absence of drug effects was observed in 5 other cells with each tested agonist.

receptor. Renzapride (BRL 24 924), cisapride, and zacopride as well as 5-MeOT (Dumuis *et al.*, 1989; Bockaert *et al.*, 1990), which are classical 5-HT₄ receptor agonists, and the newly discovered 5-HT₄ agonist BIMU 8 (Dumuis *et al.*, 1991), when used at micromolar concentrations, reduced the outward K⁺ current by 10 to 40% (Figure 5a and b, Figure 6 a–d). Among these drugs, the effect of renzapride was examined in more detail. The concentration of the agonist inducing half-maximal effect was found to be 0.1 μ M (Figure 5d, filled circles). As observed for the 5-HT response, PKI added to the intracellular medium, blocked the effect induced by renzapride at all the concentrations tested (Figure 5c and d, open circles). The non-specific protein kinase inhibitor, H7, also completely abolished the effect of renzapride (data not shown).

The only 5-HT₄ receptor antagonist so far described is ICS 205 930 which is also a potent 5-HT₃ antagonist. The affinity of this compound for 5-HT₃ and 5-HT₄ receptors is in the nanomolar and micromolar ranges, respectively (Richardson *et al.*, 1985; Dumuis *et al.*, 1989). Therefore, we first thought to use this compound as a 5-HT₄ receptor antagonist. However, at micromolar concentration ICS 205 930 blocked almost all the K⁺ current in the absence of 5-HT receptor agonist (data not shown), which corroborates the observations of Scholtysik (1987) and Scholtysik *et al.* (1988) in cardiac muscle. Therefore, this drug could not be used to test the 5-HT₄ nature of the effect on the K⁺ current.

An alternative was to use the azabicycloalkyl benzimidazolone derivative, DAU 6285 (Turconi *et al.*, 1990), a drug that we found to inhibit cyclic AMP production induced by specific 5-HT₄ receptor agonists in cultured colliculi neurones (Dumuis *et al.*, 1992). At a concentration of 10 μ M, DAU 6285 had no effect by its own on K⁺ currents of colliculi neurones (compare traces 1 of Figure 7a and b and traces 1 of Figure 7c and d). On the other hand, DAU 6285 (10 μ M) applied to the same preparation blocked the effects of 1 μ M renzapride (a specific 5-HT₄ receptor agonist) on the K⁺ current (Figure 7a and b). This compound also blocked the inhibition of the K⁺ current induced by 5-HT (compare Figure 7c and d). This strongly suggested that 5-HT₄ receptors were implicated in the observed 5-HT-induced effects.

Discussion

The present results show that part (40%) of the outward K⁺ current in colliculi neurones can be inhibited by activation of PKA. They also show that in these neurones 5-HT activates 5-HT₄ receptors (which are known to be positively coupled to adenylate cyclase; Dumuis *et al.*, 1988; 1989; Bockaert *et al.*, 1990) and blocks the cyclic AMP-sensitive K⁺ current.

Existence of a cyclic AMP-sensitive K⁺ current in colliculi neurones

We used forskolin as a pharmacological tool to activate directly adenylate cyclase and block the cyclic AMP-sensitive K⁺ current. In addition to its action on adenylate cyclase, forskolin directly alters the gating of voltage-dependent K⁺ channels from cardiac muscle cells (Scholtysik, 1987) and the clonal PC12 cell line (Hoshi *et al.*, 1988), but at concentrations 5–10 fold higher than the one used here. Therefore, it seems unlikely that this unspecific effect of forskolin may have interfered with the cyclic AMP-dependent inhibition of the K⁺ current. Moreover, inhibition of the K⁺ current observed in the presence of 8-bromo-cyclic-AMP must have resulted from cyclic AMP-dependent processes solely. This clearly demonstrated that part of the outward K⁺ current in colliculi neurones was cyclic AMP-sensitive.

Our results show that PKA was involved in 5-HT₄-mediated inhibition of K⁺ current in colliculi neurones. However, we do not know, as is the case with other K⁺ channels, whether the modulation is due to direct phosphorylation of

Figure 5 Renzapride (Renz), a 5-HT₄ receptor agonist, like 5-hydroxytryptamine (5-HT), decreased the K⁺ current in a cyclic AMP-dependent manner. Currents were evoked as in Figure 1. (a and c) Trace 1 is the control current obtained in 2 different cells. Trace 2 is obtained in the presence of renzapride (1 μ M), without (a) and with (c) protein kinase inhibitor (PKI, 1 μ M) in the recording pipette solution. (b) Current-voltage relations obtained in one cell, in the absence (●) and in the presence of renzapride (○). (d) Dose-response curve for renzapride obtained under normal intracellular recording medium (●, *n* = 10) and in the presence of PKI in the recording pipette (○, *n* = 10). Each point represents mean with s.e.mean shown by vertical bars.

Figure 6 Various 5-HT₄ agonists inhibited the K⁺ current in colliculi neurones, as 5-hydroxytryptamine (5-HT) and renzapride did. Currents were evoked according to the protocol in Figure 1. (a, b, c and d) Trace 1 is the control recording obtained in 4 different cells. Trace 2 is obtained from the same cells, in the presence of cisapride (2 μ M, a), BIMU8 (2 μ M, b), zacopride (10 μ M, c) and 5-methoxytryptamine (10 μ M, d), respectively. Similar results were obtained for each drug in 4 other cells.

Pharmacology of the 5-HT-induced inhibition of the K⁺ current

The PKA-dependence of the 5-HT-mediated effect on the K⁺ current suggests involvement of a 5-HT receptor that was positively coupled to adenylate cyclase. Such a 5-HT receptor does not correspond to any of the 5-HT receptor subtypes previously described in the CNS and confirms the existence

Figure 7 The effects of 5-hydroxytryptamine (5-HT) and renzapride on the K⁺ current were antagonized by DAU 6285, a novel 5-HT₄ receptor antagonist. Currents were evoked as in Figure 1. (a) Traces 1 and 2 were obtained in the same cell, in the absence and presence of 5-HT (1 μM), respectively. (b) Currents were recorded from the same cell as in (a), in the presence of DAU 6285 (10 μM) alone (trace 1) and in the presence of DAU 6285 with 5-HT (1 μM) (trace 2). (c and d) A similar protocol, except that 5-HT was replaced by renzapride (1 μM) and, was applied to the same cell as in (a) and (b). Similar results were obtained with both agonists in 5 other cells.

of the newly described 5-HT₄ receptor subtype in colliculi neurones (Dumuis *et al.*, 1988; 1989; Bockaert *et al.*, 1990). Several lines of evidence support this conclusion. First, the 5-HT-induced decrease in K⁺ current was insensitive to concentrations of methiothepin, ketanserin and ondansetron sufficient to completely block all 5-HT₁, 5-HT₂ and 5-HT₃ receptor subtypes, respectively. Second, RU 24 969, 5-CT or 2-CH₃-5-HT which are active at 5-HT₁ and 5-HT₃ receptors, respectively, but not at the 5-HT₄ receptor subtype (Dumuis *et al.*, 1991), did not affect the K⁺ current studied in colliculi neurones.

Third, two families of compounds are now classical agonists of the 5-HT₄ receptor subtype. One family consists of substituted indol derivatives including 5-substituted tryptamines such as 5-HT and 5-MeOT (Dumuis *et al.*, 1988; Bockaert *et al.*, 1990); the other family comprises the 2-methoxy-4-amino-5-chloro-substituted benzamide derivatives such as renzapride, cisapride and zacopride (Dumuis *et al.*, 1989). A newly discovered family of potent 5-HT₄ agonists is constituted by the azabicycloalkyl benzimidazolone derivatives such as BIMU 8 (Dumuis *et al.*, 1991). We found here that all these compounds significantly decreased the K⁺ current in colliculi neurones, suggesting a 5-HT₄ receptor-mediated effect.

Moreover, in the case of renzapride, as for 5-HT, the agonist effect on the K⁺ current was dose-dependent and was blocked by DAU 6285, a new 5-HT₄ antagonist, as revealed by cyclic AMP measurements in colliculi neurones (Dumuis *et al.*, 1992), that did not affect the K⁺ current studied on its own. This observation ruled out the possibility of an unspecific effect of the agonists on the K⁺ channels.

The 5-HT₄ nature of the 5-HT and renzapride effects was further supported by the fact that both drug effects were antagonized by intracellular application of PKI, a specific PKA inhibitor, and were potentiated by the phosphatase inhibitor, okadaic acid. We showed that PKI or okadaic acid alone did not significantly alter the basal activity of the

studied current, suggesting two conclusions. First, the current was probably not under sustained control of cyclic AMP-dependent and phosphorylation processes in the absence of agonist. Second, the 5-HT and renzapride effects were not related to some unspecific action of the drugs but rather involved activation of adenylate cyclase via 5-HT₄ receptor stimulation. We found however that PKI did not completely block the effect of 5-HT or renzapride. We do not exclude the possibility that the 5-HT₄ effect on the K⁺ current was in part directly mediated via a coupled-receptor G protein and thus independent of any PKA activity.

BIMU 8 (Dumuis *et al.*, 1991) and more classically renzapride (Turconi *et al.*, 1990) also display 5-HT₃ antagonistic activity in receptor binding assays and physiological tests. However, it seems unlikely that this property of BIMU 8 and renzapride accounted for the drug effects observed here on the K⁺ current in colliculi neurones since another potent and specific 5-HT₃ antagonist, ondansetron, did not alter the current.

Putative physiological significance of the 5-HT₄ effect in colliculi neurones

Our results indicate that the cyclic AMP-sensitive current regulated by 5-HT₄ receptors in colliculi neurones contributes in an apparent steady-state manner to the total voltage-dependent K⁺ current in these cells.

It is possible that as in *Aplysia californica* neurones (Klein *et al.*, 1982; Siegelbaum *et al.*, 1982; Pollock *et al.*, 1985), 5-HT decreased K⁺ current in colliculi neurones by inhibiting specific 5-HT-sensitive K⁺ channels (S-K⁺ channels). Indeed, as in colliculi neurones, the action of 5-HT on S-K⁺ channels in *Aplysia californica* neurones is mimicked by cyclic AMP (Castellucci *et al.*, 1980) and is inhibited by PKI (Castellucci *et al.*, 1982). However, S-K⁺ channels in *Aplysia* neurones are not voltage-dependent (Klein *et al.*, 1982) and the pharmacology of the 5-HT effects in this preparation are probably quite different from those presently observed in colliculi neurones. More specific studies are in progress in our laboratory to characterize further the 5-HT₄-sensitive K⁺ channels in colliculi neurones.

Slowly-inactivating voltage-dependent K⁺ currents, that can be inhibited by cyclic AMP, have also been described in hippocampal pyramidal neurones. These currents participate in the Ca²⁺-activated afterhyperpolarization (AHP) that normally follows spiking activity in these cells. The currents are decreased by 5-HT application (Andrade & Nicoll, 1987) and the pharmacology of this effect corresponds to activation of a 5-HT₄ receptor subtype (Chaput *et al.*, 1990; Andrade & Chaput, 1991). Our results suggest that similar effect might occur in colliculi neurones.

In hippocampal pyramidal cells (Chaput *et al.*, 1990; Andrade & Chaput, 1991) and in *Aplysia californica* neurones (Klein & Kandel, 1980; Pollock *et al.*, 1985), depression of K⁺ current by 5-HT leads to depolarization, decrease in AHP and broadening of the action potential. In colliculi neurones, as in the other two cell types, such modifications should increase cell firing, induce Ca²⁺ influx and thereby enhance transmitter release. This is thought to be the case also in *Aplysia californica* abdominal ganglion sensory neurones following inhibition of S-K⁺ channels by 5-HT, and this would underlie sensitization of the gill-withdrawal reflex of the animal, a simple form of learning (Kandel & Schwartz, 1982). In hippocampal pyramidal cells, blockade of K⁺ currents by various pharmacological agents is responsible for a novel form of long-term potentiation of excitatory synaptic response of the neurones (Cherubini *et al.*, 1987; Aniksetjn & Ben-Ari, 1991), probably by enhancing transmitter (glutamate) release. On the basis of these results and in the view of our findings, it is tempting to suggest that the blockade of K⁺ current by 5-HT is also able to increase transmitter release and thereby enhance synaptic transmission, in colliculi neurones.

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