The effect of adrenergic drugs on serotonin metabolism in the nucleus raphe dorsalis of the rat, studied by in vivo voltammetry

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The serotonin (5-HT) and norepinephrine (NE) system participate in the control of behavioural functions. The experiments were aimed at the question whether the NE system of the locus coeruleus interferes with the 5-HT activity of the nucleus raphe dorsalis and of which receptors are possibly involved. The α₁- and β-adrenoceptor agonists methoxamine and isoproterenol, as well as a high dose (600 µg/kg i.p.) of the α₁-adrenoceptor agonist clonidine, increased extraneuronal 5-hydroxyindoleacetic acid (5-HIAA) levels in the nucleus raphe dorsalis as measured by in vivo voltammetry. In contrast, a low dose (60 µg/kg i.p.) of clonidine and the α₂-, α₁- and β-adrenoceptor antagonists, prazosin, piperoxane, and atenolol, reduced the 5-HIAA concentration. In the locus coeruleus, the origin of NE projections to the nucleus raphe dorsalis, clonidine decreased whereas piperoxane enhanced extracellular 3,4-dihydroxyphenylacetic acid (DOPAC), an index of NE metabolism in the locus coeruleus. The results suggest that 5-HT neurotransmission in the nucleus raphe dorsalis is stimulated by the NE system of the locus coeruleus and that adrenoceptor drugs may affect 5-HT neuronal activity in addition to NE neurotransmission.

5-HT (5-hydroxytryptamine, serotonin); Norepinephrine; Nucleus raphe dorsalis; Locus coeruleus; Voltammetry (in vivo); (Rat)

1. Introduction

The serotonin (5-HT) neurotransmitter system is thought to be involved in behavioural functions and dysfunctions such as anxiety, aggression, the sleep-waking cycle, arousal, and depression (Jacobs et al., 1990; Trulson and Jacobs, 1979; Wauquier and Dugovic, 1990), which are also influenced by adrenoceptor drugs. The α₂-adrenoceptor agonists, e.g. clonidine, display anxiolytic properties, similarly to the 5-HT₁A agonists, 8-hydroxy-2-(di-n-propylamino)-tetraline (8-OH-DPAT), buspirone, and ipsapirone (Unnerstall et al., 1984; Ourish et al., 1986). Hypothermia, induced by 8-OH-DPAT, and hypermotility, induced by the 5-HT₁B agonist RU-24969, can be reduced by repeated treatment with β-adrenoceptor agonists (Frances and Simon, 1986). In addition, depression which is thought to be associated with – among others – dysfunction of 5-HT transmission may be treated with β-adrenoceptor agonists (Hallberg et al., 1981). It appears that in these events the norepinephrine (NE) transmitter system participates in the control of 5-HT release and that a direct innervation of the raphe nuclei by NE projections may be involved in the regulation of 5-HT activity (Maura et al., 1982).

In fact, the nucleus raphe dorsalis, which contains the highest density of 5-HT cell bodies, receives a large NE input from the locus coeruleus. Baraban and Aghajanian (1981), using electron microscopic autoradiography, demonstrated a direct innervation of the 5-HT-containing cells in the nucleus raphe dorsalis by NE terminals.

Since α₁-, α₂- and β-receptors were found to be present in the nucleus raphe dorsalis (Smith and Gallagher, 1989; Unnerstall et al., 1984; Pazos et al., 1985) we investigated the effect of adrenoceptor agonists and antagonists on the 5-HT system in the nucleus raphe dorsalis. In vivo voltammetry was used to analyze changes of extracellular 5-hydroxyindoleacetic acid (5-HIAA) levels as a measure of 5-HT metabolism. Depending on the experimental protocol extracellular 5-HIAA may be a good indicator of 5-HT release (Cespuglio et al., 1986). However, Crespi et al. (1990) provided evidence that neuronal 5-HT activity and 5-HT release are not necessarily correlated with 5-HT metabolism. Since extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) was shown to be a good index of the metabolic activity of NE neurons (Quintin et al., 1986) this metabolite was measured by in vivo voltam-
metry to follow the effect of agonists and antagonists for the NE autoreceptor in this nucleus.

2. Materials and methods

2.1. Animals and implantation of electrodes

2.1.1. Chronic experiments

The experiments on the effect of pargyline, 5-hydroxytryptophan (5-HTP), and the α- and β-adrenoceptor ligands, clonidine, piperoxane, prazosin, and isoproterenol, in the nucleus raphe dorsalis were performed in freely moving rats as follows. Wistar WU rats (Savo-Ivanovas, Kisslegg, Germany) of 300 ± 50 g weight were used for these experiments. In vivo voltammetry was performed as described by Cespuglio et al. (1984, 1986) using carbon multifibre electrodes (50 µm) to detect extracellular 5-HIAA. The electrodes were implanted chronically under chloral hydrate anesthesia (500-600 mg/kg i.p.) by means of a David-Kopf stereotaxic instrument. The following coordinates were used (Paxinos and Watson, 1982): anterior 5.2 mm, lateral ± 0 mm, vertical 5.7 mm, 45° angle from the perpendicular. The upper incisor bar was set 3 mm below the interaural line. The rats were then left to recover for 10-14 days with a 12/12-h light/dark cycle with free access to food and water. To verify the final position of the electrode at the end of the experiment, the area around the working electrode was destroyed by an electrolytic lesion (5 V, 20 s). The lesioned site was verified by light microscopy on serial coronal sections (thickness 20 µm) stained with cresyl violet.

2.1.2. Acute experiments

All voltammetric analyses in the locus coeruleus and the measurements in the nucleus raphe dorsalis after methoxamine and atenolol administration were performed under acute conditions in anesthetized rats. The animals were kept for at least 14 days under a 12/12-h light/dark cycle prior to the experiment. The experiments started 4 h after the lights were turned on. The rats were anesthetized with chloral hydrate (500-600 mg/kg i.p.) and kept under anesthesia until the end of the experiment. When measurements were to be performed in the nucleus raphe dorsalis, the rats were pretreated with allopurinol (20 mg/kg i.p.) to inhibit uric acid production (Cespuglio et al., 1986). Carbon monofibre electrodes (12 µm diameter and 500 µm long) were implanted in the locus coeruleus using the following coordinates (Gonon et al., 1983): anterior −0.7 mm to the interaural line, lateral 1.3 mm and vertical 6.1 mm below the cerebellar surface. The upper incisor bar was 10 mm below the interaural line. For 5-HIAA analyses in the nucleus raphe dorsalis the above mentioned coordinates (see ‘Chronic experiments’) were used. The position of the electrode was verified histologically at the end of the experiments.

2.2. Voltammetric recordings

The carbon fibre electrodes were pretreated electrochemically and tested in vitro as described by Gonon et al. (1981) and Cespuglio et al. (1984). Differential pulse voltammetry (Biopulse, Tacussel, France; Polarorecord E 506, modified for in vivo voltammetry, Metrohm, Germany) versus an Ag/AgCl reference electrode was used to measure extraneuronal 5-HIAA and DOPAC oxidation currents. Measuring pulse: amplitude 50 mV, duration 60 ms. Scan rate: 10 mV/s. Sweep from 0 to +500 mV for recordings in the nucleus raphe dorsalis and from −250 to +250 mV for recordings in the locus coeruleus. Voltammetric signals at +100 and +280 mV were characterized pharmacologically as DOPAC and 5-HIAA, respectively, as described by Buda et al. (1983), and Cespuglio et al. (1986). Voltammetric recordings were performed every 5 or 10 min and started 30 min before application of the drug. Injections were always performed at time 0. The results are expressed as means ± S.D. and were analyzed using an analysis of variance (ANOVA) with repeated measurements, for P < 0.05 compared to the control.

2.3. Reagents and drugs

All drugs were obtained from Sigma (Deisenhofen, Germany), except for chloral hydrate which was from Merck (Darmstadt, Germany), and clonidine and piperoxane from RBI-Biotrend (Köln, Germany). Allopurinol was dissolved in 0.1 M NaOH, all other drugs were dissolved in saline and administered i.p. at the doses indicated.

3. Results

Under chronic conditions the 5-HIAA signal in the nucleus raphe dorsalis of freely moving untreated rats (fig. 1A) remained constant over the detection period whereas, under acute conditions, a 25% decrease was observed after 4 h (data not shown). The identity of the voltammetric signal in the nucleus raphe dorsalis as 5-HIAA was validated by the oxidation current at +280 mV, inhibition of monoamineoxidase (amine: oxygen oxidoreductase (deaminating) EC 1.4.3.4) (MAO) with pargyline, and increased 5-HT synthesis after 5-HTP administration. After pargyline (fig. 1A), the voltammetric 5-HIAA signal disappeared completely after 1 h, due to MAO inhibition. The application of 5-HTP led to a dramatic increase of the signal, with a maximum of 400% when tested 30 min after
administration (fig. 1B). Accordingly in acute experiments the DOPAC signal of the locus coeruleus was identified by its oxidation current at +100 mV and by the reduction of the peak after MAO inhibition with pargyline (data not shown). Since neuronal NE activity can be mediated by $\alpha_2$-autoreceptors being located presynaptically on NE neurons the effect of the $\alpha_2$-agonist, clonidine, on the DOPAC and 5-HIAA levels was measured in the locus coeruleus and the nucleus raphe dorsalis, respectively. In accordance with published data for a wide range of concentrations, clonidine (600 $\mu$g/kg i.p.) reduced DOPAC in the locus coeruleus whereas the same concentration increased the 5-HIAA level in the nucleus raphe dorsalis (figs. 2, 3A).

However, a low dose of clonidine (60 $\mu$g/kg i.p.) reduced the extracellular 5-HIAA concentration in the nucleus raphe dorsalis (fig. 3A). An effect the inverse of that of clonidine was observed with the $\alpha_2$-antagonist, piperoxane. This drug increased the DOPAC signal in the locus coeruleus and reduced the 5-HIAA level in the nucleus raphe dorsalis (figs. 2, 3B). Besides $\alpha_2$-receptors, $\alpha_1$- and $\beta$-receptors have been identified in the nucleus raphe dorsalis. Hence the effects of $\alpha_1$- and $\beta$-agonists and antagonists on 5-HIAA levels were studied. The $\alpha_1$-agonist, methoxamine, produced an increase of the 5-HIAA signal (fig. 4), but this effect was rather weak when compared to that of clonidine (fig. 3A). The $\alpha_1$-antagonist, prazosin, induced a decrease of the 5-HIAA level in the nucleus raphe dorsalis to about 60% of the control (fig. 4). An increase of the 5-HIAA signal was also obtained with the $\beta$-agonist, isoproterenol, with a peak in the initial 20 min and lasting until 80 min after injection (fig. 5). In contrast, the $\beta$-antagonist, atenolol, drastically decreased the 5-HIAA signal in the nucleus raphe dorsalis, with a maximal reduction 3 h after injection.

4. Discussion

The participation of both the 5-HT and the NE system in a number of physiological functions and pathophysiological dysfunctions suggests that these two
systems are somehow coupled. To mention one example: during arousal, besides the well-known activation of the NE system the 5-HT neurotransmitter system is also stimulated, as was shown with acoustic and immobilization stress (Trulson and Jacobs, 1979; Wesemann et al., 1990). The role of the activated 5-HT system during arousal is not yet clear. Alternative explanations would be that 5-HT contributes to the arousal reaction or that the enhanced 5-HT activity avoids hyperarousal (Trulson and Jacobs, 1979). Interaction of the NE and the 5-HT system which apparently are both involved in the arousal reaction must be assumed in both cases. As a contribution to the possible mode of interaction of the two aminergic systems in vivo voltammetry was used to monitor the changes of extracellular DOPAC and 5-HIAA concentrations which were induced by administration of $\alpha_1$, $\alpha_2$ and $\beta$-receptor agonists and antagonists.

The voltammetric catechol signal in the locus coeruleus was identified by pargyline and L-DOPA as DOPAC (results not presented), as was first shown by Gonon et al. (1983) and Buda et al. (1983). Cedarbaum and Aghajanian (1977) reported that the firing rate of the NE cells declines after application of clonidine. In
accordance with published data (Gonon et al., 1983) we found that the \( \alpha_2 \)-agonist, clonidine, reduced whereas the \( \alpha_2 \)-antagonist, piperoxane, increased the DOPAC signal in the locus coeruleus. As was shown previously clonidine reduces the DOPAC levels dose dependently at all concentrations tested (Tibirica et al., 1989). Since the DOPAC levels in the locus coeruleus are an index of the metabolic activity of NE neurons (Quintin et al., 1986) the correlation of electrophysiological and biochemical findings suggests that the reduction of the DOPAC levels observed after clonidine reflects the decreased firing rate of NE neurons and a reduced NE release in the locus coeruleus. The voltammetric signal in the nucleus raphe dorsalis was identified as 5-HIAA by means of pargyline and 5-HTP (fig. 1). Complete disappearance of the 5-HIAA signal induced by pargyline was obtained in chronic experiments only more than 10 days after implantation of the electrodes. The peak declined to about 30% of the control 5 days after implantation. This remaining part of the signal may be due to uric acid (Cespuglio et al., 1986).

In the nucleus raphe dorsalis, the effects of clonidine differed according to the dose used (60 and 600 \( \mu \)g/kg i.p.). While the high dose of clonidine increased the voltammetric 5-HIAA signal, the low dose decreased the signal in the nucleus raphe dorsalis (fig. 3). This is in agreement with the report of Aslanian and Renaud (1989) that the voltammetric signal in the nucleus raphe magnus, which also receives NE projections from the locus coeruleus, is decreased by a low dose (50 \( \mu \)g/kg) of clonidine. Since a high clonidine dose (400 \( \mu \)g/kg i.p.) increases and a low dose (50 \( \mu \)g/kg i.p.) decreases the firing rate of 5-HT neuron in the nucleus raphe dorsalis (Svensson et al., 1975), the increased 5-HIAA levels found in the nucleus raphe dorsalis after a high clonidine dose might reflect an increased 5-HT release in this nucleus, though it does not prove it. Crespi et al. (1990) provided evidence that under specific conditions, neuronal electrical activity, 5-HT release and metabolism might be regulated differently. It is plausible that clonidine in low concentrations may act mainly presynaptically whereas higher doses may also act postsynaptically. Along this line, behavioural studies have shown that low and high doses of clonidine act differently. Clonidine at higher concentrations was found to be a sleep-inducing substance (Monti, 1982), whereas lower doses significantly reduced slow wave sleep (SWS) and rapid eye movement (REM) sleep (Hilakivi, 1983).

A dose-dependent inversion of the piperoxane effects on the neuronal 5-HT system has not been reported suggesting that this \( \alpha_2 \)-antagonist acts primarily at postsynaptic receptors. Hence, the reduction of the 5-HIAA signal following piperoxane administration is not unexpected and is in agreement with previous observations of Marwaha and Aghajanian (1987) that piperoxane reduces the firing rate of 5-HT neurons in the nucleus raphe dorsalis. In addition these authors showed that \( \alpha_2 \)-antagonists such as prazosin also reduced the 5-HT neuronal firing. This finding was confirmed biochemically in the present study as prazosin reduced the extraneuronal 5-HIAA concentrations in the nucleus raphe dorsalis (fig. 4). Consequently, the \( \alpha_2 \)-receptor agonist, methoxamine, increased 5-HIAA levels in the nucleus raphe dorsalis. This effect was long-lasting albeit weaker than that of a high dose of clonidine. Since we have shown that low doses of clonidine inhibit 5-HT metabolism in the nucleus raphe dorsalis, this observation suggests that methoxamine, which is sometimes used to antagonize peripheral effects of low clonidine doses, may also antagonize central effects after administration of low doses of clonidine.

The \( \beta \)-adrenergic system interferes with neuronal 5-HT activity as well. Isoproterenol increased, whereas the \( \beta \)-antagonist, atenolol, reduced, the 5-HIAA signal in the nucleus raphe dorsalis. Antagonists of \( \beta \)-adrenoreceptors reduce 5-HT synthesis (Hallberg et al., 1982) and antagonize 5-HT-induced behavioural effects such as the 5-HTP-induced head-twitch response (Deakin and Green, 1978) and 5-HT induced sleep (Weinstock et al., 1977). The \( \beta \)-adrenoreceptor agonist, salbutamol, increases brain 5-HT synthesis (Hallberg et al., 1981). The metabolic and behavioural changes are probably at least partly caused by the effect of adrenoreceptor agonists and antagonists on neuronal 5-HT activity and/or metabolism as was now shown by in vivo voltammetry. The antidepressant effects of some \( \beta \)-agonists may in fact be associated with an increased neuronal 5-HT activity.

These results obtained by in vivo voltammetry show that agonists of the neuronal NE system, in particular the \( \alpha_2 \)-agonist, methoxamine, the \( \alpha_2 \)-agonist, clonidine, in a high dose and the \( \beta \)-agonist, isoproterenol, were capable of enhancing 5-HT metabolism in the nucleus raphe dorsalis. Opposite effects were observed with adrenoreceptor antagonists. Since the NE innervation of the nucleus raphe dorsalis descends from the locus coeruleus, our data suggest a close functional interaction of both the 5-HT and the NE neuronal systems. Therefore, treatment with adrenoreceptor agonists or antagonists must always induce profound changes in both NE and 5-HT neurotransmission and correlated behaviour. Though still speculative at the moment the suggestion can be made that the inverse effects obtained with a low and a high dose of clonidine are consistent with the assumption that the 5-HT system of the nucleus raphe dorsalis is depressed by low NE activity of the locus coeruleus whereas a high NE firing rate activates the 5-HT metabolism of the nucleus raphe dorsalis to avoid hyperarousal.
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