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Non-steroidal anti-inflammatory agents, tolmetin and sulindac, inhibit liver tryptophan 2,3-dioxygenase activity and alter brain neurotransmitter levels

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Abstract

Hepatic tryptophan 2,3-dioxygenase (TDO) is one of the rate-limiting enzymes in tryptophan catabolism and plays an important role in regulating the physiological flux of tryptophan into relevant metabolic pathways. In this study, we determined the effect of the non-steroidal anti-inflammatory agents, tolmetin and sulindac, on rat liver TDO activity and the subsequent changes in the hippocampal and striatal neurotransmitter levels. The amount of melatonin produced by the pineal gland was also measured using high performance liquid chromatography (HPLC). Treatment of rats with tolmetin or sulindac (5 mg/kg/bd for 5 days) significantly inhibited liver TDO activity. The results show that whilst tolmetin and sulindac increase serotonin levels in the hippocampus, these agents also significantly reduce dopamine levels in the striatum. Tolmetin, but not sulindac, increased the amount of melatonin produced by the pineal gland. The results of this study suggest that whilst tolmetin and sulindac may be beneficial for patients suffering from depression, these agents also have the potential to induce adverse effects in patients suffering with neurological disorders such as Parkinson's disease.

Keywords: Tolmetin; Sulindac; Tryptophan 2,3-dioxygenase; Serotonin; Dopamine; Melatonin; Parkinson's disease; NSAIDS

Introduction

Hepatic tryptophan 2,3-dioxygenase (TDO) is the rate-limiting enzyme in the oxidative breakdown of tryptophan to kynurenine in the body. Its activity determines the relative tryptophan flux into the serotonergic and kynurenine pathways. Hepatic TDO is specific for tryptophan as the substrate (Hayaishi, 1980).

Tryptophan is hydroxylated to 5-hydroxytryptophan by tryptophan hydroxylase, which is present in high concentrations in the pineal gland, and is the rate-limiting step in the synthesis of 5-hydroxytryptamine (5-HT) (Lovenberg et al., 1968). It is the amount of tryptophan available and not the enzyme activity that influences the rate of the reaction (Deguchi and Barchas, 1972; Bensinger et al., 1974). In the pineal gland there is sufficient aromatic amino acid decarboxylase, which decarboxylates 5-hydroxytryptophan to produce 5-HT.

Serotonin is an important regulator in a wide range of physiological and biochemical processes in vertebrates. Serotonin is involved in the maintenance of circadian rhythmic functions, blood pressure regulation, acts as a neurotransmitter at central and peripheral regions (Kema et al., 2000), and is known to act as its own trophic factor (Mohanakumar et al., 1995). Alterations in brain serotonin levels are known to result in mood disorders, particularly depression (van Praag, 1982). Serotonin can be *N*-acetylated to form *N*-acetyl serotonin, in a reaction catalyzed by the enzyme *N*-acetyltransferase. In most circumstances this is the rate-limiting step in melatonin synthesis (Klein et al., 1997). The 5-hydroxy group of *N*-acetylserotonin is *O*-methylated in the presence of hydroxyl-*O*-methyltransferase to form melatonin.

In the last several years, melatonin, the chief secretory product of the pineal gland (Reiter, 1991), has been found to be both a direct free radical scavenger and an indirect antioxidant (Hardeland et al., 1995; Hardeland, 1997; Reiter et al., 1995;

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Reiter et al., 1997), in addition to its function as a neurohormone. Because of these actions, melatonin has been pharmacologically tested for its ability to reduce oxidative damage in a variety of experimental neurological processes and has been found to be highly effective in this respect (Reiter et al., 1997; Reiter, 1998). Melatonin is both lipid (Costa et al., 1995) and water soluble (Shida et al., 1994), although its lipid solubility is greater and has the ability to traverse almost every organ in the body (Reiter, 1995). It has been demonstrated to be a powerful antioxidant and free radical scavenger (Tan et al., 1993) and reduce oxidative damage in the central nervous system (Reiter, 1998).

Neuroinflammation has been implicated in the pathogenesis of neurological disorders such as Alzheimer's and Parkinson's disease (McGeer et al., 1988; Castano et al., 1998; Langston et al., 1999; Cicchetti et al., 2002). Although non-steroidal anti-inflammatory drugs (NSAIDS) may offer neuroprotection via anti-inflammatory action, other mechanisms may also be involved at the neurotransmitter level (Mohanakumar et al., 2000; Sairam et al., 2003). Any drug used for the treatment of whichever disease should not aggravate the underlying pathology. In this study, we determined the effects of tolmetin and sulindac, commonly used NSAIDS for pain, inflammation, arthritis and rheumatoid arthritis, on brain neurotransmitter levels in the hippocampus and striatum. The effect of these NSAIDS on rat liver TDO activity and biogenic amines such as serotonin, dopamine and their metabolites as well as the amount of melatonin produced by the pineal gland were determined.

Materials and methods

Chemicals

Tolmetin, sulindac, melatonin, L-tryptophan, hematin, dopamine (DA), serotonin, 5-hydroxy-3-indoleacetic acid (5-HIAA) and dihydroxyphenyl acetic acid, (DOPAC), phosphoric acid (PA) and octane sulphonate (OSA) were purchased from Sigma Chemicals Company, St Louis, MO, U.S.A. Trichloroacetic acid (TCA), triethylamine (TEA) and perchloric acid ($HCLO_4$) were obtained from Saarchem, Johannesburg, ethylenediamine tetraacetic acid (EDTA) was purchased from HOLPRO Analytics, (PTY) LTD, Johannesburg, South Africa. HPLC grade, acetonitrile (ACN), methanol (MeOH) and chloroform were purchased from BDH Laboratory Supplies, Poole, England. Minimum Essential Medium (MEM) was purchased from Highveld Biologicals, South Africa. All other reagents were of the highest quality available.

Animals

Adult male rats of the Wistar strain, weighing between 250–300 g were purchased from South African Institute for Medical Research (Johannesburg, South Africa). The animals were housed in a controlled environment with a 12-h light: dark cycle, and were given access to food and water ad libitum. The Rhodes University animal ethics committee approved protocols for the experiments.

Methods

Treatment regimes

Animals were divided into three groups A, B and C. Group A served as the control while groups B and C received either tolmetin or sulindac at a dose of 5 mg/kg/bd for 5 days. On the 6th day animals were killed by neck fracture. The livers were removed, perfused with cold normal saline and stored at −70 °C until use. The hippocamppi and striatum were dissected from the brain, frozen in liquid nitrogen and stored at −70 °C until use. The pineal gland from each rat was removed and incubated for 24 h at 37 °C in 50 μl of MEM that was supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and amphotericin B (2.5 μg/ml) in a sterile humidified incubator.

Liver tryptophan 2,3-dioxygenase activity

The activity of the enzyme was determined according to a modified method previously described by Badawy and Evans (1975). The livers were thawed, chopped into fine pieces and homogenised with 60 ml 140 mM KCl/2.5 mM NaOH using a glass–teflon hand held homogenizer. The homogenate was sonicated for a period of 2 min at 30 s intervals for complete release of enzymes from the cells. Finally 0.2 M sodium phosphate buffer pH 7.0 was used to make up the volume required to yield a 10% w/v homogenate. The entire procedure, where possible, was conducted on ice. An aliquot of 15 ml homogenate was added to a flask containing 12.5 ml water. An aliquot of haematin (100 μl) at a final concentration of 2 μM (Badawy and Evans, 1975) was added to samples that were used to determine the total activity of the enzyme. This was stirred for 1 min to allow for the activation of the enzyme. Finally, 2.5 ml of 0.03 M L-tryptophan was added to all flasks and gently stirred. The assay was conducted in triplicate. Aliquots of 3 ml of the assay mixture was transferred to test tubes, stoppered under carbogen and incubated for 1 h at 37 °C in an oscillating water bath. The enzyme activity was determined in the absence and presence of haematin in order to determine the activity of the holo- and apoenzymes of TDO. The apoenzyme in isolation is inactive but in the presence of haematin becomes fully active. The holoenzyme activity was measured in the absence of haematin while the total activity was measured in the presence of added haematin. The reaction was terminated with the addition of 2 ml of 0.9 M TCA to the reaction mixture and incubated for 2–4 min. The mixture was filtered through a Whatman no. 1 filter paper. Filtrate (2.5 ml) was added to 1.5 ml of 0.6 M NaOH and vortexed. The kynurenine present in the solution was measured at 365 nm spectrophotometrically using the molar extinction coefficient of kynurenine: $\varepsilon = 4540\ M^{-1}.cm^{-1}$. The blank consisted of 2 ml TCA and 1.5 ml NaOH. The TDO activity was expressed as nmol/mg protein/h. Protein estimation was performed using the method described by Lowry et al. (1951).

High performance liquid chromatography (HPLC)–electrochemical evaluation of biogenic amines

Instrumentation and chromatographic conditions. Samples were analyzed on an isocratic HPLC coupled to an electrochemical