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Short communication

Melatonin affords protection against gastric lesions induced by ischemia-reperfusion possibly due to its antioxidant and mucosal microcirculatory effects

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Abstract

Melatonin, a pineal hormone, is known to scavenge oxygen free radicals and to be present in the gut but little is known about its role in the protection of gastric mucosa against the damage accompanied by a marked increase in these radicals. This study was designed to determine the effects of melatonin on the formation of acute gastric lesions induced by ischemia-reperfusion and, for comparison, by a topical irritant such as 100% ethanol. It was found that pretreatment with melatonin at a dose of 5 mg/kg given intragastrically reduced significantly gastric lesions induced by ischemia-reperfusion and this was accompanied by a reduction in free radicals in the blood and by attenuation of the fall in gastric blood flow. In contrast, melatonin failed to affect acute gastric lesions induced by 100% ethanol. We conclude that melatonin is capable of protecting gastric mucosa from the damage caused by ischemia-reperfusion and that this action is mediated, at least in part, by limitation of the generation of free radicals and by attenuation of the fall in gastric blood flow.

Keywords: Melatonin; Free radical; Cytoprotection; Ischemia-reperfusion; Gastric ulceration; Gastric blood flow

1. Introduction

Melatonin, a pineal hormone, has attracted attention predominantly due to its role in the control of circadian rhythmic organization (Cassone, 1990; Lewy et al., 1992; Mahe and Chevalier, 1995; Waldhauser et al., 1988) but the unique property of this hormone is its ability to protect the integrity of various cells due to its antioxidant effect, in part due to the scavenging of oxygen free radicals (Hardeland et al., 1993; Reiter et al., 1995). Melatonin undergoes an iron-porphirin-mediated reaction with hydroxyl radicals, resulting in the formation of the indolyl cation which then is believed to scavenge superoxide anion radicals.

Melatonin has also been detected in high concentrations in the gut (Heuther, 1994), but no information is available regarding the possible effects of this hormone on gastric mucosal integrity. Since ischemia-reperfusion causes gastric mucosal lesions, at least in part, due to the formation of oxygen radicals (Itoh and Guth, 1985; Kawashi et al., 1994; Smith et al., 1996), we decided to determine the influence of melatonin on gastric mucosal lesions induced by ischemia-reperfusion as well as by topical irritants such as ethanol and to assess the possible mechanism of action, including the role of the gastric mucosal circulation and the generation of oxygen radicals.

2. Material and methods

Wistar male rats, weighing 180–220 g and fasted for 24 h, were used in all studies.

2.1. Measurement of gastric acid secretion and plasma gastrin level

Gastric secretory studies were carried out on animals surgically equipped with chronic gastric fistulas. A catheter

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was placed in the carotic artery for sampling blood for measurement of plasma gastrin levels during the experiment (Brzozowski et al., 1995; Konturek et al., 1991). About 2 weeks later, the animals were placed in individual cages, the cannulas of the fistulas were opened and the stomachs were washed out with saline. The basal gastric juice was collected for five consecutive 30 min. After three 30-min control samples had been collected, the vehicle (1 ml of saline) or melatonin (dissolved in 1 ml of saline) was applied intragastrically (i.g.) and the gastric fistula was closed for a 30-min period. Then, the fistula was opened again, the stomach was drained for 5 min and this collection was discarded. The collection was continued for two 30-min periods while saline was infused s.c. at a rate of 4 ml/h throughout the experiment to replenish the fluid lost as gastric secretions. Gastric acid output was measured in each 30-min sample and expressed as mean output per 30-min period before and after the administration of melatonin or vehicle.

Blood samples (about 2 ml) obtained from the carotic artery during the secretory studies once 30 min before and once 60 min after the administration of melatonin were collected in EDTA-containing vials. The plasma was separated by centrifugation and then stored at −20°C until radioimmunoassay using gastrin antiserum 4562 (kindly donated by Prof. J.E. Rehfeld, Århus, Denmark) in a final dilution of 1:140 000. The antibody recognized G-17 and G-34 equally. The sensitivity of the gastrin measurement in the present assay was 2.5 pM/ml plasma, equivalent to that for human G-17 as described before (Konturek et al., 1990).

2.2. Production of gastric lesions

Gastric lesions were produced by ischemia-reperfusion following clamping of the celiac artery (Wada et al., 1995) and by topical irritants such as 100% ethanol (Konturek et al., 1991) in male Wistar rats weighing 180–220 g and fasted for 18 h. Briefly, the erosions induced by ischemia-reperfusion were produced by opening the abdomen and clamping the celiac artery for 30 min. The animals were then anesthetized after 1, 24 and 48 h and the abdomen was opened and the stomach was exposed to measure gastric blood flow as described below. The stomach was then removed to measure the area of gastric lesions, using computerized planimetry (Konturek et al., 1991), and blood samples were withdrawn from the vena cava inferior to determine plasma gastrin level (Konturek et al., 1990). In the case of topical irritants, 1.5 ml of 100% ethanol was administered intragastrically (i.g.) through the orogastric tube and the rats were anesthetized 1 h later to measure gastric blood flow and the area of gastric lesions (Konturek et al., 1991).

Several groups of animals with gastric lesions were used. Group A was used to examine the effects of melatonin (Sigma, St. Louis, MO, USA) on gastric lesions induced by ischemia-reperfusion. Group B was used to assess the effects of melatonin on acute gastric lesions induced by 100% ethanol. These experimental groups were treated with: (1) vehicle (saline given i.g.) followed 30 min later by ischemia-reperfusion or 100% ethanol, or (2) melatonin (5 mg/kg i.g.) followed 30 min later by ischemia-reperfusion or 100% ethanol.

2.3. Measurement of gastric blood flow

In groups of animals used in ischemia-reperfusion experiments, 1 h before gastric lesions were induced and 1, 24 or 48 h after ischemia-reperfusion, the rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and the abdomen was opened by a midline incision. The stomach was exposed and the gastric mucosal blood flow was measured by hydrogen gas (H_2) clearance, as described previously (Konturek et al., 1991). The gastric blood flow was measured at three adjacent sites in the mid portion of the anterior wall of the oxyntic gland area of the stomach. The mean values of three recordings were calculated before and after ischemia-reperfusion without or with pretreatment with melatonin, and expressed as percent changes from the control value recorded in rats with an intact stomach before ischemia-reperfusion. In rats exposed to ethanol, the gastric blood flow was measured 1 h after the administration of this irritant. After these gastric blood flow measurements, the stomach was removed, opened along the greater curvature and the area of gastric lesions was determined using planimetry (Morphomat, Carl Zeiss, Berlin, Germany) by two investigators unaware of the treatment given.

2.4. Assessment of blood chemiluminescence

In ischemia-reperfusion experiments, the venous blood was taken from the vena cava just after measurement of the gastric blood flow. Blood was collected in EDTA-containing vials and was used either for chemiluminescence study or for radioimmunoassay of plasma gastrin. Whole blood chemiluminescence was measured by a modification of a procedure described by Tono-Oka et al. (1983). 20 μl blood was mixed with 400 μl of 2 mM luminol (5 amino-2,3-dihydro-1,4-phthalizinedione; Sigma) in Krebs-Ringer buffer with Mg^{2+} and Ca^{2+} in a measuring vial. Then 20 μl of latex beads (0.8–1.0 μm diameter, ca. 2×10^{10}/ml, PAN, Kraków, Poland) was added and vials were placed in the measuring chamber of a Multi-Biolumat (Berthold, Vienna, Austria). The reaction was carried out at 37°C and the chemiluminescence was continuously recorded. The results were calculated as integral (cc = cumulative counts) of the response in a 10-min period.

Table 1
Effect of graded doses of melatonin (1.2–10 mg/kg i.g.) on gastric acid output and plasma levels of gastrin in rats with chronic gastric fistulas

Type of test	Acid output (μmol/30 min)	Gastrin (pg/ml)
Basal	112 ± 9	72 ± 7
Melatonin (mg/kg i.g.)		
1.2	118 ± 12	82 ± 6
2.5	126 ± 9	90 ± 12
5.0	92 ± 8	98 ± 11 [a]
10.0	84 ± 7 [a]	102 ± 12 [a]

Results are means ± S.E.M. of 6 experiments with 6–8 rats per group.
[a] Vehicle-treated rats.

2.5. Statistics

The results are reported as means ± S.E.M. Statistical significance was determined by analysis of variance and, where appropriate, by the unpaired Student's *t*-test, a value of $P < 0.05$ being considered significant.

3. Results

3.1. Effects of melatonin on gastric acid secretion and plasma gastrin level

Table 1 shows the effect of i.g. application of vehicle (saline) or various doses of melatonin given in a volume of 1 ml on gastric acid and plasma gastrin levels in rats with a chronic gastric fistula. The i.g. application of melatonin in gradually increasing doses from 1.2 to 5.0 mg/kg failed to affect significantly the gastric acid outputs, but at a higher dose (10.0 mg/kg) it caused a small but significant decrease in acid secretion. This decrease was accompanied by a small but significant elevation in plasma gastrin levels.

3.2. Effects of melatonin on acute gastric lesions and free radicals in blood of rats subjected to ischemia-reperfusion or administration of 100% ethanol

Table 2 shows the effects of i.g. treatment with melatonin at a constant dose of 5 mg/kg on the formation of acute gastric mucosal lesions, gastric blood flow and blood chemiluminescence in rats subjected to ischemia reperfusion at various time intervals (1, 24 and 48 h). In vehicle-treated animals, clamping of the celiac artery caused almost complete stagnation of gastric blood flow. These results have not been included. One hour after the removal of the clamp, the blood flow was decreased to about 50% of the value recorded in intact rats. The blood flow was still reduced 24 and 48 h after the termination of the ischemia-reperfusion experiment. Gastric lesions started to appear in the oxyntic mucosa immediately after the removal of the arterial clamp but reached significantly higher values after 1 h, to achieve the maximum at 24 h. The whole blood chemiluminescence was almost tripled after ischemia-reperfusion and then tended to decline after 24 and 48 h. The pretreatment with melatonin significantly increased the gastric blood flow and reduced the mucosal lesions and the chemiluminescence at all time intervals after ischemia-reperfusion.

The mucosal lesions induced by 100% ethanol were not significantly affected by the pretreatment with melatonin at a dose of 5 mg/kg, although the lesion area tended to decrease (Table 2). The application of 100% ethanol resulted in a reduction in gastric blood flow to about 25% and this was slightly but significantly attenuated by pretreatment with melatonin. Ethanol failed to affect significantly the blood chemiluminescence, and pretreatment with

Table 2
The area of gastric lesions, gastric blood flow (GBF), and blood chemiluminescence in intact rats and in animals examined after 1, 24 or 48 h of ischemia-reperfusion given vehicle (saline) or melatonin (5 mg/kg i.g.)

	Lesion area (mm^2)	GBF (ml/100 g · min)	Chemiluminescene (cc/10 min)
Intact (vehicle) rats	0	59 ± 6	1.2 ± 0.3
Melatonin in intact rat (5 mg/kg i.g.)	0	69 ± 10	1.0 ± 0.4
Ischemia-reperfusion (control)			
1 h	22.4 ± 2.5 [a]	26 ± 4 [a]	3.6 ± 4.4 [a]
24 h	38.3 ± 5.2 [a]	31 ± 5 [a]	2.2 ± 0.6 [a]
48 h	36.1 ± 5.2 [a]	38 ± 4 [a]	1.9 ± 0.3 [a]
Ischemia-reperfusion + melatonin (5 mg/kg i.g.)			
1 h	10.0 ± 1.6 [b]	37 ± 5 [b]	2.1 ± 0.4 [b]
24 h	16.0 ± 2.9 [b]	46 ± 4 [b]	1.6 ± 0.2 [b]
48 h	10.8 ± 1.6 [b]	56 ± 6 [b]	1.4 ± 0.2 [b]
100% Ethanol (control)	90.4 ± 12.4	15 ± 3	1.9 ± 0.4
Melatonin (5 mg/kg i.g.) + 100% ethanol	79.6 ± 21.7	28 ± 4 [b]	1.6 ± 0.3

Values are means ± S.E.M. for 8–10 rats.
[a] Significant change as compared to vehicle control.
[b] Significant change as compared to the values obtained in vehicle-treated rats without melatonin.

melatonin did not influence the blood chemiluminescence in rats administered 100% ethanol.

4. Discussion

This study provides evidence that melatonin applied intragastrically protects the gastric mucosa against the damage induced by ischemia-reperfusion but fails to affect gastric lesions provoked by topical irritants such as 100% ethanol. This protective action of intragastric melatonin against ischemia-reperfusion-induced gastric lesions was accompanied by a reduction in the blood chemiluminescence, suggesting that this protection depends, at least in part, upon the scavenging of oxygen free radicals. This finding is in agreement with previous observations that ischemia-reperfusion enhances the generation of oxygen free radicals (Smith et al., 1996) and that melatonin is a very efficient neutralizer of these radicals (Reiter et al., 1995).

Since melatonin and its binding sites have been detected in the gastrointestinal tract (Heuther, 1994; Lee and Pang, 1993), it was of interest to determine whether this hormone contributes to the integrity of the gastric mucosa. Our finding that the protective action of melatonin against the ischemia-reperfusion-induced injury was accompanied by an attenuation of the post-ischemia fall in gastric blood flow suggests that a vascular factor may be implicated in this protection. This is supported by the observation that melatonin failed to influence the mucosal lesions provoked by 100% ethanol, even though gastric blood flow was dramatically reduced (by about 75%) as compared to that of intact mucosa; blood flow remained decreased after the administration of melatonin. The mechanism of the elevation of mucosal microcirculation by melatonin during ischemia-reperfusion is not known, but it could be due to the release of local vasoactive substances, such as prostaglandins or nitric oxide, which are recognized mediators of the gastroprotection provided by other hormones (Whittle et al., 1990; Konturek et al., 1995). Our secretory studies demonstrate that i.g. applied melatonin produces a rise in plasma gastrin with concomitant inhibition of gastric acid secretion. As gastrin is known to exhibit gastroprotective activity (Konturek et al., 1995), it is not excluded that the protective action of melatonin could be attributed, at least in part, to the release of gastrin, but this requires further studies.

The major finding of this study is that melatonin suppresses the generation of blood free radicals (as measured by chemiluminescence of whole blood, which are known to play a major pathogenic role in ischemia-reperfusion-induced gastric lesions (Smith et al., 1996). As the main source of free radicals in the blood is circulating neutrophils, it is reasonable to assume that melatonin prevents or reduces the activation of these cells and, thus, attenuates the oxidative action of these radicals on the gastric mucosa as well as on the gastric microcirculation. Previous studies (Cho et al., 1989) showed that melatonin decreases the aggravation of gastric mucosal injury and gastric blood flow caused by the administration of serotonin in the ex vivo stomach preparation, suggesting that melatonin produced locally in the gastric wall (Cho et al., 1989; Bubenik and Pang, 1994) acts as a modulator of the action of serotonin in the gastrointestinal tract. In another report (Pentney and Bubenik, 1995), melatonin was found to protect against lipopolysaccharide-induced damage and this effect also has been attributed to the antioxidant activity of this hormone. Further studies are needed to determine whether melatonin is involved in physiological mechanisms to maintain gastric mucosal integrity and whether it cooperates with other cytoprotective substances in this respect.

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