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EFFECT OF MELATONIN ON INTESTINAL FLUID ABSORPTION IN THE DOMESTIC CHICKEN (*GALLUS DOMESTICUS*)

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SUMMARY

A possible effect of Melatonin (MEL) on intestinal fluid transport in the chicken has been studied. Fluid transport was measured in terms of the rate of serosal fluid transfer (SFT), gut fluid uptake (GFT), and mucosal fluid transfer (MFT) using the everted gut sac method. Five hundred microgram of MEL was administered to each of the 8-week old pullets daily for 100 days. Significantly increased (P<0.001) mean SFT was observed in the jejunum, while in the ileum, SFT was significantly decreased (P<0.001). Mean GFU and MFT were significantly higher in the control than in MEL- treated birds, both in the jejunum (GFU, P<0.001; MFT, P<0.001), and in the ileum (GFU, P<0.02, MFT, P<0.001). Melatonin may therefore be involved in the modulation of fluid transference across the wall of the intestine.

Keywords: Melatonin, everted sac, intestine, fluid transport, and chicken.

RESUME

L' effet possible de la melatonine (MEL) sur le transport intestinal des fluides chz les poussins a ete etudie. Ce transport e'tait mesure' en termes de taux de fluide serique, absorption intestinale et transfer mucosal. Cinq cent microgrammes de MEL etaient administres a chaque poussin (age de 8 semaines) par jour pendent cent jours. Une croissance considerable (P<0,001) de la moyenne de fluide seriale fut enregisfree dans le jejunum alors que dans l' ileum le taux etait negligeable (p>0,001). les faux moyens de GFU et MFT du groupe temoin etaient considerablement superieurs a celuidu groupe traite, avec MEL, que ce soit dans le jejunum (GFO P<0,001 Mfa, P<0,001) et U ileum GFU P<0,02 Mfa P<0,001). En conclusion, la melatonine pourait etre impliquee dans la regulation des fluides a travers les parois de clintestin.

Although Melatonin is primarily the major secretion of the pineal gland, several extra pineal sources of Melatonin have been identified in virtually all-animal species including man. Such extra pineal sources include the retina (Osol *et al*, 1984, Peinado *et al*, 1990), Harderian gland (Menedez- Palaez, 1988) and the gastro intestinal tract (GIT) (Gern and Kern, 1983, Ralph, 1984). Melatonin has also been shown to affect the functions of organs such as the testes, skin, eye, ovary, liver and pituitary in all of which melatonin receptors have been located (MacPhee *et al*, 1975; Tamarkin *et al*, 1977; Cohen *et al*, 1978 and Cardinale *et al*, 1979). There is evidence from the literature indicating that melatonin directly affects the properties of the intestinal mucosa (Chan *et al*, 1998). Also melatonin production by the cell of the GIT appears to be independent of the pineal gland, since pineal ablation does not affect the concentration of melatonin in the tissues of the GIT (Ralph, 1984). The enterochromaffin cells are sparsely located in the gastric mucosa, are fairly prevalent in the small and large intestine. These cells perform the endocrine functions of the gut tissue and secrete histamine, and probably melatonin (Norman, 1987). It is doubtful however, if such melatonin performs endocrine function, but rather local actions (Underwood *et al*, 1987). It has been proposed that melatonin may serve a protective function to the cells of the GIT, probably by acting as a scavenger of free radicals (Konturek *et al*, 1997), or via the action of it's mucosal receptors (Chan *et al*, 1998).

Opinion on the role of melatonin in intestinal transport and barrier function has not attained a consensus among workers in this area of interest. It was the objective of the present study to contribute to a suggestion of a possible role for melatonin on intestinal transport by investigating its effect on intestinal fluid transport in the chicken.

MATERIALS AND METHODS

Experimental Animals.

Twelve healthy 8-week-old pullets (Nera strain) domestic chicken were used. They were purchased from a commercial poultry farm (X-Towere Farms, Ibadan, Nigeria), and housed in the experimental animal housing unit of the Department of Veterinary Physiology and Pharmacology, University of

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Ibadan, Nigeria. Birds were randomly divided into two groups viz.; the MEL- treated and the control groups, each group consisting of six birds.

Birds in each group were housed in a deep litter communal housing unit (cubicles), each measuring 1.5 X 1.5 X 0.3 m. lighting in the housing unit was controlled and arranged to simulate the environmental photoperiod which is roughly 12 hours of light and 12 hours of darkness. Light on was at 07.00 h off at 19.00 h. Birds were given commercial poultry feeds (growers mash until point of lay, and thence layers mash (Bendel Feeds and Flour Mills Ltd., Nigeria), and water ad- libitum. Birds were dewormed with piperazine dihydrochloride, single dose immediately on arrival and 5 weeks before commencement of experiments.

Administration of melatonin and preparation of everted sac of the intestine.

Melatonin (Sigma, Poole Dorset, U. K.) was dissolved in 10% ethanol in distilled water (v: v) to a concentration of 10 µg/µl. each bird in the experimental group received 50 µl (500 µg of MEL) of the solution daily for 100 days, while the control hens received 50 µl of ethanol buffer solution. It has been suggested that orally administered melatonin may undergo enterohepatic circulation (English *et al*, 1987). Plasma melatonin in rats and man undergo hepatic hydroxylation to 6 hydroxymelatonin which is then excreted in the urine and faeces as a sulphate conjugate, acetyl- methoxy tryptamine 6-sulphate (aMT6S) (Kopin *et al*, 1961; Kennaway *et al*, 1982).

At the end of treatment period, each animal was starved for 24 hours and then killed by decapitation. The lower abdomen was incised and the whole of the small intestine was excised. The everted sac technique of Wilson *et al*, (1954) was employed. Briefly, the intestinal lumen was washed with Kreb- Ringer solution and the mesentery carefully cut away from the intestine. For eversion a stainless steel rod (300 X 15 mm) was pushed slightly into the ileal end of the intestine, held in place with a thread tied round the intestine. The rod was then pushed inside the intestine, pulling the ileal end until it appeared at the duodenal end. The proximal end of the gut was then rolled over the rod, thus completing the eversion.

Four segments each of 10-cm length were taken from the small intestine of each animal and made into sacs. Two sacs were made each from the jejunal as well as ileal regions. The sacs were numbered I- IV from the jejunal to the ileal regions. One end of each was tightly occluded with a piece of thread. Two ml of the serosal fluid (freshly prepared 28-mM glucose Krebs bicarbonate solution) was introduced in the serosal-lined cavity and the other of the segment occluded with a piece of thread, thus forming a sac.

Treatment of sacs following their preparation was effected as earlier described by Oyewale *et al*, (1987). Methods of calculating the results were as described by Parsons *et al*, (1958), and the terms used in describing these were as used by Barry *et al*, (1961). Intestinal fluid transport was determined by measuring the following terms; Serosal fluid transfer (SFT), the amount of taken from the mucosal region and transferred to the serosal region. Gut fluid uptake (GFU), the amount of fluid taken from the mucosal region but which was retained within the gut wall and mucosal fluid transfer (MFT), the sum of the two.

The results were expressed as means and standard deviation, while comparison of the means were made using the student' t-test. A probability value of 0.05 or below was considered as significant.

RESULTS

The mean values observed for SFT, GFU and MFT in the melatonin- treated and control chicken are shown in table 1.In the melatonin- treated birds, mean SFT value decreased from 240 ± 30 µl/g/30 min in the jejunum, to 140 ± 30 µl/g/30 min in the ileum (P<0.001). Although an increase was observed in the control group for SFT from 130 ± 70 µl/g/30 min in the jejunum to 200 ± 100 µl/g/30 min in the ileum, this is not a significant change. In the jejunum, a significant difference (P<0.01) was observed for SFT between the MEL- treated birds and the controls (Table 1). In the ileum, SFT value was 200 ± 100 µl/g/30 min in the control, while in the MEL- treated birds, the corresponding was 140 ± 30 µl/g/30 min. These values are not significantly different from each other.

Gut fluid uptake in the ileum of MEL- treated birds (380 ± 30 µl/g/30 min), was much higher than the corresponding value of 70 ± 10 µl/g/30 min in the jejunum (P<0.01). Also, in the control chicken, GFU was 170 ± 70 µl/g/30 min in the jejunum. This value increased to 400 ± 170 µl/g/30 min in the ileum. This represents a significant difference (P<0.01). The difference in the mean GFU between the control and MEL- treated chicken is also significant (170 ± 70 vs 70 ± 10 µl/g/30 min respectively (P<0.01). In the ileum however, no significant difference was observed for the GFU value between the control and MEL- treated chicken (Table 1). In MEL- treated chicken, the MFT value of 450 ± 50 µl/g/30 min observed in the ileum, was significantly different (P<0.001) from the corresponding value

100 µl/g/30 min observed in the jejunum. The situation in the control birds was similar to EL- treated, in that MFT value of 260 ± 100-µl/g/30 min in the jejunum is significantly lower) than the ileal value of 550 ± 30-µl/g/30 min. However, while no significant difference was d for the MFT value in the jejunum between the two groups of bird in the ileum, mean MFT nificantly higher (P<0.001) in the control birds compared to MEL-treated ones (Table 1).

SD) of serosal fluid transfer (SFT), gut fluid uptake (GFU) and mucosal fluid transfer (MFT) in the melatonin and the control domestic chicken.

	SFT (ul/g/30min)		GFU (ul/g/30min)		MFT (ul/g/30min)	
	Jejunum n = 12	Ileum n = 12	Jejunum n = 12	Ileum n = 12	Jejunum n = 12	Ileum n = 12
Treated	240 ± 30	140 ± 30	70 ± 100	380 ± 30	160 ± 100	460 ± 50
61	130 ± 70	200 ± 100	170 ± 70	400 ± 70	260 ± 100	550 ± 30

esents number of segments used
nificant difference (P < 0.001) between figures on the same row and within the same column
nificant difference (P < 0.0i) between figures on the same row and within the same column

CUSSION

As shown in the result section of the present study and in Table 1, chronic oral administration of resulted in generalised decrease in intestinal fluid absorption as determined by SFT, GFU and T. However, in the jejunum, a higher SFT value was observed in the MEL-treated chicken relative to control.

This also confirms previous studies that the everted sac is suitable for the study of transference of d, electrolyte, and metabolites in several animal species (Wilson *et al*, 1954; Rowdon *et al*, 1973; ainoya, 1982). The present study also confirms regional difference in intestinal absorption of fluid h greater rate at the posterior (ileal) segment, than at the middle (Jejunal) segment (Mainoya, 1982). inhibition of intestinal fluid observed in thisstudy in MEL- treated chicken, maybe a function of e receptors of melatonin which have been located in the of several animal species (Poon *et al*, 1997; han *et al*, 1998). ^{125}Iodomelatonin-binding site have been shown to widely distribute in the lamina opia of the proventriculus, duodenum, ileum and in the muscle layer of the caecum in domestic icken (Poon *et al*, 1997). A role has been proposed for melatonin acting through its receptors in the gulation of chloride ions in human colon (Chan *et al*, 1998). Rice *et al*, (1982), Curran *et al* (1962), ave proposed that water transport by the intestine is a passive process resulting from active solute nsport. It is possible that while MEL regulates electrolyte secretion or absorption in the gut, it will so regulate fluid transference and as shown in this study, it results in reduced intestinal fluid ansport. In the present study, the observed reduction in gut fluid transport in MEL- treated chicken annot be explained on the proposed activity of MEL as a scavenger of oxygen free radicals in the gut. their study, Konturek *et al*, (1972) have demonstrated a dose-dependent effect of MEL and L-yptophan in reduction of stress-induced gastric lesions accompanied by reduced blood free radicals nd attenuation of gut blood flow. Since it has been demonstrated earlier that the rate of intestinal bsorption bears a direct relationship with the rate of blood flow (Levin, 1966). The observed result in is study probably suggests a reduction and not an enhanced blood flow to the gut as observed by onturek *et al* (1997).

Although in the present study, MEL was administered orally, the dose given and the duration of treatment suggest that the observed effect may be a central action through the brain. Melatonin acting entrally has been shown to affect the production of a number of reproductive hormones, which are nown to influence intestinal fluid transport. For instance MEL suppresses ovarian oestrogen secretion in human and rodents (MacPhee *et al*, 1975, Reiter, 1980).

In a study by Ogwuegbu (1987), increased intestinal fluid absorption observed in laying fowl was ttributed to high circulating level of oestrogen in the fowl. The converse may also be true, that is, reatment /factors which cause reduction in circulating oestrogen may also cause reduced intestinal fluid transference. Durotoye and Sanni (1999) have demonstrated inhibitory effect of oestrogen secretion by the birds.

ONCLUSION: Results of the present study indicate that MEL administration causes reduced intestinal fluid transference in female domestic chicken. We suggest that this effect of MEL may be a direct effect acting through its receptors which have been located in the gut, or through a central effect n the brain by affecting the secretion or blood levels of hormones like oestrogen.

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