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SHORT COMMUNICATION

THE INFLUENCE OF SEROTONIN ON THE MITOTIC RATE IN THE COLONIC CRYPT EPITHELIUM AND IN COLONIC ADENOCARCINOMA IN RATS

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SUMMARY

1. The mitotic rate in the crypts of Lieberkühn of the descending colon and in dimethylhydrazine-induced adenocarcinomata of the descending colon of rat was measured using a stathmokinetic technique.

2. Intraperitoneal injection of a small dose (10 μg/kg) of serotonin resulted in an increase in the tumour cell mitotic rate.

3. Blockade of serotonin receptors by 2-bromolysergic acid diethylamide and depletion of tissue serotonin levels following injection of DL-6-fluorotryptophan both result in a decrease in the tumour cell mitotic rate.

4. Treatment with serotonin, 2-bromolysergic acid diethylamide and DL-6-fluorotryptophan were all without effect on the colonic crypt cell mitotic rate.

Key words: adenocarcinoma, colon, mitosis, serotonin.

INTRODUCTION

Serotonin, a remarkably uniquitous biogenic amine, has been shown to promote cell division in a wide variety of cell types, both animal and plant (Hedinger & Langemann, 1955; Niaussat *et al.*, 1958). Amongst the mammalian cell types whose division is stimulated by serotonin are hepatocytes (MacDonald *et al.*, 1959), basal epidermal cells (Mann, 1967), fibroblasts (Norrby, 1973) and jejunal crypt cells (Tutton, 1974). It has recently been shown that in both colonic crypt epithelium and in colonic adenocarcinoma cell proliferation is greatly retarded during the phase of generalized biogenic amine depletion which follows injection of reserpine (Tutton & Barkla, 1976a). In the present communication the effect of injection of either serotonin or serotonin antagonists and of specific depletion of serotonin following injection of DL-6-fluorotryptophan on the mitotic rate in the colonic crypt epithelium and in chemically-induced colonic adenocarcinoma is reported.

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METHODS

Male Sprague-Dawley rats were fed Clark King Nu-pig pellets and tap water *ad libitum* and housed at 21-24°C with artificial light from 07.00 to 21.00 hours and darkness from 21.00 to 07.00 hours. Rats were given weekly injections of 1,2-dimethylhydrazine (Aldrich Chemical Co. Inc., Milwaukee, Wisconsin) at a dose of 21 mg/kg, s.c., as previously described (Druckery *et al.*, 1967; Tutton & Barkla, 1976b). After 21 weeks the dimethylhydrazine injections were discontinued and following an interval of 2–8 weeks the animals were used in the experiments described below.

Estimation of tissue serotonin content

Serotonin content of five specimens of descending colon (full thickness of wall) and in colonic carcinomata were estimated using a fluorescence assay based on the technique of Wise (1967). Similarly measurements were made in another group of five rats 4 h after the injection of DL-6-fluorotryptophan. The statistical significance of differences between the serotonin levels in control and DL-6-fluorotryptophan treated tissues was assessed using Student's *t*-test.

Estimation of mitotic rates

All rats were injected with vinblastine sulphate (Velbe, Elly Lilly Co.; 4 mg/kg at 12.00 hours) and were killed by decapitation at times ranging from 12.45 to 16.00 hours. Counts of metaphase and of non-metaphase cells in colonic crypts and in colonic adenocarcinomata were made at a magnification of x 1250 and metaphase indices were calculated and corrected for sectioning and geometric artefacts as previously described (Tutton & Barkla, 1976b).

Graphs of true metaphase index *vs* duration of vinblastine treatment were then constructed for each experimental group of tissues having mitoses blocked for periods of 0.75 to 4.0 h. The regression coefficient for each of the graphs was then calculated using the method of least squares; this calculated value represents the rate at which cells enter metaphase and has the units of mitoses/cell per h. The statistical significance of apparent differences between the values of the regression coefficient for different experimental groups of tissue was estimates by analysis of variance (Bliss, 1967).

Initially cell proliferation was studied in the colonic crypts of nine dimethylhydrazine-treated rats and in five dimethylhydrazine-induced adenocarcinomata.

In order to evaluate the role of tissue serotonin levels in the control of cell division in the colonic crypts and in colonic adenocarcinomata five dimethylhydrazine-treated rats were injected intraperitoneally with DL-6-fluorotryptophan (Sigma Chemical Co., St Louis, U.S.A.) at a dose of 300 mg/kg given at 10.00 hours. Cell proliferation in the colon of these animals was then studied during the period 12.00 to 16.00 hours on the same day. DL-6-fluorotryptophan specifically inhibits the enzyme tryptophan hydroxylase (McGeer, Peters & McGeer, 1968), leading to a lowering of serotonin levels in a wide variety of tissues (Peters, 1971). Ten dimethylhydrazine-treated rats were injected with serotonin (in the form of 5-hydroxytryptamine creatinine phosphate), four of these receiving a dose of 100 μg/kg and six receiving a dose of 10 μg/kg. The serotonin injections were given at 12.00 hours and all doses were calculated in terms of the free base.

To test the effect of blockade of serotonin receptors four dimethylhydrazine-treated rats

were injected with 2-bromolysergic acid diethylamide (BOL 148, Roche, Basel, Switzerland) at a dose of 0.25 mg/kg given every 2 h commencing at 12.00 hours.

RESULTS

In rats partially depleted of serotonin following injection of 6-fluorotryptophan and in animals whose serotonin receptors were blocked by treatment with bromolysergic acid diethylamide cell proliferation proceeded at its normal rate in the crypt epithelium but essentially ceased in the colonic tumours. Conversely, injection of a small dose of serotonin (10 μg/kg) caused an increase in the mitotic rate in the tumour but not in the crypt epithelium. Numerical values for mitotic rates and tissue serotonin levels are given in Table 1.

Table 1. Effect of DL-6-fluorotryptophan, 2-bromolysergic acid diethylamide and serotonin on the mitotic rate and on the serotonin content in colonic crypt epithelium and in colonic adenocarcinomata

Treatment	Mitotic rate (mitoses/cell per h)				Serotonin content (μg/g)			
	Colonic crypts		Colonic carcinoma		Descending colon		Colonic carcinoma	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
Nil (control)	0.024	0.006	0.025	0.007	3.9	0.4	4.3	0.5
DL-6-fluorotryptophan	0.016*	0.004	0.002‡	0.005	1.6**	0.2	2.2††	0.5
Serotonin (10 μg/kg)	0.029*	0.011	0.055§	0.010	Not estimated			
Serotonin (100 μg/kg)	0.025*	0.006	0.024†	0.004	Not estimated			
2-bromolysergic acid diethylamide	0.029†	0.003	0.005¶	0.002	Not estimated			

* Not significantly different from control values ($P > 0.25$, analysis of variance, d.f. = 14/1).
† Not significantly different from control values ($P > 0.5$, analysis of variance, d.f. = 13/1).
‡ Significantly lower than control value ($P < 0.005$, analysis of variance, d.f. = 10/1).
§ Significantly higher than control value ($P < 0.25$, analysis of variance, d.f. = 11/1).
¶ Significantly lower than control value ($P < 0.01$, analysis of variance, d.f. = 9/1).
** Significantly lower than control value ($P < 0.01$, Student's t, d.f. = 8).
†† Significantly lower than control value ($P < 0.05$, Student's t, d.f. = 10).

DISCUSSION

Previous results from this laboratory have shown that cell proliferation in both colonic crypt epithelium and colonic adenocarcinoma is dependent upon biogenic amines (Tutton & Barkla, 1976b). However, cell division in the crypt epithelium differs from that in the tumour in being stimulated by α-adrencoceptor agonists and inhibited by α-adrenoceptor blockade as well as by noradrenaline depletion (Tutton & Barkla, 1977). Results in this study show another important difference between cell division in colonic tumours and that in their antecedent epithelium, namely that tumour cell division, unlike that in the crypt epithelium, is promoted by injection of serotonin and is inhibited by blockade of serotonin receptors as well as by serotonin depletion.

Results of serotonin assays extend the findings of Peters (1971) by showing that DL-6-fluorotryptophan is effective for depleting serotonin in both colon and in colonic tumours.

In both descending colon and in colonic tumours the percentage depletion of serotonin was similar to that reported by Peters (1971) for the small intestine.

Differences between the biogenic amine requirements of the colonic crypt epithelium and colonic tumour epithelium may prove very important in anti-cancer chemotherapy. Blocking α-adrenoreceptors and simultaneously stimulating serotonin receptors may, by transiently decreasing the ratio between cell proliferation rates in normal and in neoplastic cells, increase the efficacy of those antineoplastic drugs whose specificity is for rapidly dividing cells.

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