THE EFFECT OF PAIN STIMULATION ON THE MITOTIC ACTIVITY OF THE EPITHELIUM OF THE SMALL INTESTINE IN HYPOPHYSECTOMIZED RATS

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The stimulating effect of pain on the mitotic activity in the epithelium of the crypts of Lieberkühn in the small intestine has been reported by several workers after experiments on rats [2, 3, 9]. In other mammalian tissues, also characterized by a high level of mitotic activity (epithelium of the tongue and cornea, epidermis of the ear), the effect of pain is to cause a reactive inhibition of cell division, attributed by most workers to stimulation of the secretion of adrenalin [1, 5-8]. This view of the important role of adrenalin in the development of the reactive inhibition of mitotic activity is based on considerable experimental evidence, and may now be considered firmly established.

Meanwhile the problem of the mechanism of stimulation of the mitotic activity in the mucous membrane of the small intestine by pain has not been specially investigated. Since the increase in the number of cell divisions in the epithelium of the crypts of Lieberkühn occurs only when the pain is intense, such as, for example, a burn in the region of the thigh [9], division of the sciatic nerve [2], or resection of the tip of the tongue [3], causing stress, it may be assumed that this stimulation is a manifestation of the general adaptation syndrome and is directly dependent on the system of the pituitary and adrenal cortex. In order to verify this assumption, experiments were conducted on hypophysectomized animals.

EXPERIMENTAL METHOD

The investigation was carried out on 25 mongrel albino male rats weighing 160-180 g, from which the pituitary was removed by the usual parapharyngeal method. The same operation was performed on control rats, but the pituitary was left in situ. Three weeks after hypophysectomy, the sciatic nerve was divided in 6 control and 7 experimental rats; this was regarded as a strong pain stimulus. All the rats fasted for 8 h before the experiment began. The animals were sacrificed 40 min after division of the sciatic nerve, by decapitation; 6 control rats and 6 hypophysectomized rats not undergoing pain stimulation were sacrificed at the same time. During postmortem examination of the animals (which lasted from 8 h 20 min to 10 h), attention was paid to the body weight and to the weight of the testes and adrenals, from which the completeness of hypophysectomy was judged. Only those animals in which the pituitary was completely removed were used in the experiment.

To study the mitotic activity, an area of the small intestine taken at a distance of 9 cm from the pylorus was fixed in Bouin's fluid. Mitoses were counted in phases in 50 crypts, divided longitudinally in transverse paraffin-wax sections cut through the intestine in a thickness of 7 μ stained with hematoxylin-eosin. The intensity of mitotic activity was judged by the number of mitoses present in 1000 cells. The number of cells in all the crypts in which mitoses were counted was determined. This procedure was necessitated by the fact that in previous work [4] we found a statistically significant difference between the number of cells to a crypt in the control and experimental animals. The results of the present counts confirmed previous findings of a reduction in the number of cells (by an average of 15) in the crypts of the small intestine 3 weeks after hypophysectomy.

Because of this shortening of the crypts in the hypophysectomized animals, the question arises whether the distribution of mitoses in the crypts is changed after hypophysectomy. To answer this question, the number of cell divisions occurring in the superior (adjoining the villus), middle, and inferior thirds of the crypt, including its base, was counted separately in the control and hypophysectomized rats. The numerical results were analyzed statistically by the Fisher – Student method.

EXPERIMENTAL RESULTS

The results of the counting of the cell divisions, showing their distribution by phases and the localization of the mitoses along the length of the crypt in the control and hypophysectomized animals, are shown in Tables 1 and 2.

The results in Table 1 show that control rats undergoing no manipulation other than the operation (similar in nature to hypophysectomy) had an average mitotic activity of 64 mitoses per 1000 cells, or 4 mitoses per crypt, and that 63.7% of the total number of mitoses were found in the middle third of the crypt, 31.5% in the inferior third, and only 4.8% in the superior third of the crypt. The mitotic activity in the control rats rose significantly 40 min after division of the sciatic nerve to 80.3 per 1000 cells, or 4.9 per crypt. The corresponding values of P were 0.002 for the mitotic coefficient and 0.009 for the number of mitoses per crypt. Analysis of the distribution of mitoses by phases showed that the increase in the number of cell divisions in the epithelium of the small intestine after division of the sciatic nerve took place on account of an increase in the number of the first two phases of mitosis.

The figures relating to the mitotic activity in the epithelium of the crypts of Lieberkühn of the hypophysectomized animals showed that the mean number of mitoses per crypt was 2.8, i.e., much below the figure in the control animals (the difference was statistically significant: P=0.001), although this difference was due to the difference in the number of cells per crypt in the control and hypophysectomized animals and it disappeared when the mitotic coefficient was calculated; in the hypophysectomized rats this was 58.7, and was not different from that in the control rats (difference not statistically significant: P=0.2). The topographical distribution of mitoses in the crypt of the hypophysectomized rats was similar to that in the crypt of the control animals, i.e., most mitoses were found in the middle and inferior thirds of the crypt and only 4.0% in the superior third.

TABLE 1. Effect of Division of the Sciatic Nerve on the Mitotic Activity in the Crypts of Lieberkühn of the Intestine

Experiment No.	Control									
			distribution of mitoses							
			by phase				topographical			
							third of crypt			
	No. of mitoses per 1000 cells	No. of mitoses per crypt	prophase	metaphase	anaphase	telophase	inferior	middle	superior
1	64,09	4,6	15	142	16	61	72	154	8
2	62,5	3,8	10	144	8	28	61	118	11
3	67,4	3,9	14	128	18	34	48	135	11
4	61,3	3,7	17	129	11	29	59	116	11
5	58,01	3,9	24	103	23	49	68	120	7
6	71,4	4,5	19	147	17	44	81	136	10
Mean	64,1 ±1,97	4,0	16,5 (8%)	132 (64,5%)	15,5 (7,6%)	40,8 (19,6%)	64,8 (31,8%)	129 (63,4%)	9,6 (4,7%)

TABLE 2. Effect of Division of the Sciatic Nerve on the Mitotic Activity in the Crypts of Lieberkühn of the Intestine

Experiment No.	Hypophysectomy								
			distribution of mitoses						
			by phase				topographical		
							third of crypt		
	No. of mitoses per 1000 cells	No. of mitoses per crypt	prophase	metaphase	anaphase	telophase	inferior	middle	superior
1	74,29	3,3	17	98	14	36	75	86	4
2	63,23	2,9	6	97	17	25	49	92	4
3	54,89	2,7	14	78	19	23	45	84	5
4	61,29	3,2	9	96	21	33	56	99	4
5	49,4	2,4	6	73	13	27	55	58	6
6	49,03	2,2	10	72	7	22	40	61	10
Mean	58,7 ±3,5	2,8	10,3 (7,4%)	85,7 (61,8%)	15 (10,9%)	27,6 (19,9%)	53,3 (38,4%)	80 (57,6%)	5,5 (4%)

It will be seen from Tables 1 and 2, and from the figure that 40 min after division of the sciatic nerve the number of dividing cells in the crypts of Lieberkühn did not increase in the hypophysectomized animals, in contrast to the controls, but a significant (P=0.004) decrease was observed in the number of cell divisions to an average level of 42.3 per 1000 cells, or of 2.08 per crypt. This fall in the number of dividing cells in the crypts of the hypophysectomized animals after division of the sciatic nerve resulted from a decrease in the number of the first two phases of mitosis and was regarded as evidence of inhibition of mitotic activity.

Hypophysectomy thus had no significant effect on the restorative phase of physiological regeneration of the intestinal epithelium, as shown not only by the mainanence of the level of mitotic activity in the hypophysectomized animals by comparison with the controls, but also by the constancy of the topographical distribution of the mitoses. This fully confirmed our earlier findings [5]. Meanwhile the present research gave further evidence that intense pain has a stimulating action on the mitotic activity in the epithelium of the small intestine in normal rats. In hypophysectomized rats, on the other hand, pain was found not to stimulate mitotic activity. The fact that after hypophysectomy there was no increase in the number of cell divisions after pain stimulation was evidently associated with the loss of essential stimuli rather than with loss of reactivity by the intestinal epithelium.

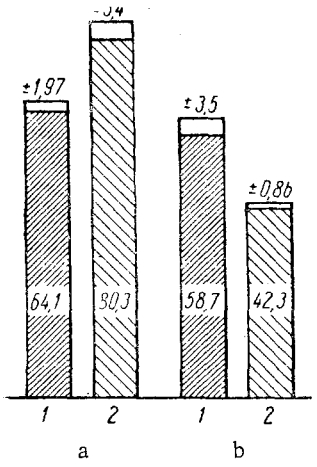
In a previous report we presented data indicating that 3 weeks after hypophysectomy considerable atrophy of the mucous membrane of the small intestine takes place, although the level of mitotic activity is unchanged and its diurnal rhythm is maintained. Consequently, the intestinal epithelium of hypophysectomized animals also remains capable of increased mitotic activity at certain times of the day.

in Control Rats

Division of sciatic nerve								
		distribution of mitoses						
		by phase				topographical third of crypt		
No. of mitoses per 1000 cells	No. of mitoses per crypt	prophase	meta-phase	ana-phase	telo-phase	inferior	middle	superior
92,7	6,2	33	198	27	50	82	206	20
70,7	4,5	37	132	17	39	77	137	11
77,3	4,8	31	114	15	51	88	134	21
72,5	4,3	31	135	10	40	86	123	7
85,4	5,0	30	167	15	38	86	147	17
83,1	5,1	36	165	13	43	97	146	14
80,3 ±3,3	4,9	33 (13,3%)	156,8 (62,9%)	16,1 (7,2%)	43,5 (17,4%)	86 (34,4%)	148 (59,5%)	15 (6%)

of Hypophysectomized Rats

Hypophysectomy + division of the nerve								
		distribution of mitoses						
		by phases				topographical third of crypt		
No. of mitoses per 1000 cells	No. of mitoses per crypt	prophase	meta-phase	ana-phase	telo-phase	inferior	middle	superior
40,7	2,08	10	60	16	18	29	67	8
42,8	2,1	11	62	13	19	34	69	6
42,2	2,08	5	69	13	17	26	66	12
45,6	2,1	4	61	17	23	31	69	5
40,2	2,04	11	55	12	21	32	70	0
42,3 ±0,86	2,08	8,2 (7,9%)	60,1 (58,5%)	14,2 (13,8%)	20,2 (19,6%)	30,4 (29,1%)	68,2 (65,0%)	6,2 (5,9%)



Changes in the number of mitoses (in ‰) in the crypts of Lieberkühn of control (a) and hypophysectomized (b) rats not otherwise manipulated (1) and 40 min after division of the sciactic nerve (2).

The suggestion that the system of the pituitary and adrenal cortex plays an important part in the increase in the number of cell divisions in the intestine after pain stimulation is thus apparently confirmed.

A fact of particular interest is that in the hypophysectomized animals 40 min after division of the sciatic nerve mitotic activity in the epithelium of the crypts of Lieberkühn became inhibited. This inhibition was possible due to an increase in the secretion of adrenalin. The cells of the adrenal medulla are known to maintain the normal secretion of adrenalin in the absence of the pituitary [10]. Reports have recently appeared indicating that inhibition of mitotic activity in the intestinal epithelium of mice under the influence of adrenalin may be found in experiments in which the duration of the mitotic cycle is measured. These facts suggest that the increased secretion of adrenalin by hypophysectomized animals in response to pain stimulation may lead to inhibition of cell division in the epithelium of the small intestine in the absence of regulating influences from the pituitary.

SUMMARY

A study was made of the effect produced by pain stimulus on the mitotic activity in the Liberkühn crypt epithelium of the small intestine in control and hypophysectomized rats. 40 minutes after division of the sciatic nerve there occurred stimulation of the cellular mitosis in the Lieberkühn crypt epithelium in control animals and inhibition of mitotic activity in the intestinal epithelium of hypophysectomized rats.

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