MODIFICATION OF RAT INTESTINAL MUSCARINIC CHOLINERGIC RECEPTORS BY IONIZING RADIATION

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ABSTRACT

Evidence exists for increased fluid and chloride secretion induced by ionising radiation. Acetylcholine via stimulation of muscarinic epithelial receptors may be implicated in such increases. This study addresses the question of whether ionising radiation modifies cholinergic muscarinic receptors.
Male Wistar rats were exposed to total body gamma irradiation (8 Gy; 1.3 $Gy.min^{-1}$; ^{60}Co source). Plasma membranes were prepared from small intestinal mucosal scrapings and marker enzyme activities (sucrase, Na+/K+-ATPase) measured. Muscarinic receptor binding characteristics (Kd, Bmax) were determined using the non-selective muscarinic antagonist ^{3}H-QNB (quinuclidinylbenzilate).
Sucrase and Na+/K+-ATPase activities are maximally decreased 3 and 4 days (D3, D4) post irradiation (3 fold). Myeloperoxidase activity is markedly reduced (10 fold at D4) and is still lower than control values 21 days after irradiation (5 fold). Two ^{3}H-QNB binding sites are observed for control rats. For the high affinity site, Kd is decreased up to 7 days after irradiation with a maximum at D4 (4 fold decrease) whereas Bmax is unchanged. For the low affinity binding site, both Kd and Bmax are reduced. The significance of these sites remains unclear.
The increase of the affinity of the high affinity binding site is in agreement with an increased response to cholinergic stimulation, and so with increased fluid and chloride secretion. These results suggest a possible implication of a dysfunction of cholinergic regulation in irradiation-induced diarrhoea. Thus it is of interest to determine the mechanisms by which radiation can modify muscarinic mucosal receptors.

INTRODUCTION

Acetylcholine, released from parasympathetic nerve endings, has a major role in the control of intestinal function. In particular, increased stimulation of muscarinic epithelial receptors may result in fluid hypersecretion. (1). Several reports show that ionising radiation induces intestinal fluid and chloride secretion (2,3).
In addition Otterson (4) observed a decrease of the amount of acetylcholine esterase (degradative enzyme) in the mucosa after irradiation. This suggests that irradiation may alter cholinergic regulation of mucosal functions so leading to increased fluid and electrolyte transport.
This study addresses the question of whether ionising radiation modifies the characteristics of cholinergic muscarinic receptors in the mucosa of rat.

METHODS

Male Wistar rats (Laboratory C.E.R.Janvier), weighing between 280 and 300g, were exposed to whole body gamma irradiation and received a dose of 8 Gy (1.3Gy.min^{-1}, ^{60}Co source). Control rats were sham-irradiated during the same period. The small intestine was removed under anaesthesia (sodium pentobarbital: 60mg.kg^{-1}) up to 21 days after irradiation and animals euthanised following an overdose of anaesthetic. Small intestinal plasma membranes were prepared from mucosal scrapings (both ileum and jejunum were pooled for each rat) and kept at -80°C. Enzyme activities were determined by spectrophotometric assays : Sucrase (5), Na^+/K^+-ATPase (6).

Determination of muscarinic receptor characteristics was performed using a non-selective muscarinic antagonist ^{3}H-QNB (quinuclidinylbenzilate). Membrane (200µg protein) were incubated with increasing concentrations of ^{3}H-QNB ranging from 50pM to 15nM. Non specific binding was determined in the presence of atropine (50µM). Separation of bound and free ligand was by rapid filtration. The experiments were performed 1, 3, 4 and 7 days after irradiation on either irradiated or sham-irradiated rats.

Analysis of binding data was by Scatchard analysis.

Statistics: A one way Anova test was used to test populations of control rats and for enzyme activities experiments, and a one way Anova Dunnett's test for binding experiments. (ns = non statistically significant; * = $p<0.05$)

RESULTS

Determination of enzyme activities

Sucrase is an enzyme associated with the apical membrane and is essentially located on the top of the villi. The results presented in the Table 1 show that sucrase activity was greatly decreased to 17% of control values 3 and 4 days after irradiation ($p<0.05$). Nine and 21 days after irradiation the sucrase activity went back to control values (ns). In parallel, the activity of the basolateral enzyme, Na^+/K^+-ATPase was determined and it is clear that irradiation modifies the Na^+/K^+-ATPase activity with a time dependent pattern similar to the pattern observed for the sucrase. Na^+/K^+-ATPase activity was unchanged at D0 but falls to 66 and 60% of basal level 3 and 4 days after irradiation respectively ($p<0.05$), and returned to basal values at D9 (ns). At D21 a second decrease in activity of 72% is observed.

Table 1: Determination of sucrase and Na^+/K^+-ATPase activities

Day after irradiation or sham irradiation	Sucrase (U/mg protein)				Na+/K+ ATPase (U/mg protein)			
	control rats	Irradiated rats	n	p	control rats	Irradiated rats	n	p
D0 at 6hours	1.26 ± 0.12	1.02 ± 0.13	2	ns	0.98 ± 0.06	0.99 ± 0.04	2	ns
D3	(n=19)	0.22 ± 0.08	5	*	(n=19)	0.33 ± 0.14	5	*
D4		0.22 ± 0.02	4	*		0.39 ± 0.06	4	*
D9		1.01 ± 0.08	6	ns		0.73 ± 0.09	6	ns
D21		0.79 ± 0.09	4	ns		0.27 ± 0.09	4	*

Characterisation of muscarinic receptors

Analysis of 3H-QNB binding data of control rats indicated the presence of 2 binding sites, one of high affinity and low capacity and one of lower affinity but higher capacity. No difference was observed for control animals during the 7 days following the simulation of irradiation (ns) and the results obtained for all control rats were pooled.

Figure 1 summarises the results obtained for binding characteristics tested up to 7 days after an 8 Gy irradiation. The Kd of the high affinity binding site (Kd1) is decreased to 24.4% of the basal value at D4 after irradiation. This decrease is still observed at D7 (25.8, $p<0.05$). No significant change in the number of sites (Bmax1) was observed for this high affinity binding site (ns) even 4 and 7 days after irradiation. For the lower affinity binding site, both Kd (Kd2) and Bmax (Bmax2) were decreased at D4 (11.5% for Kd and 14.1% for Bmax). Seven days after irradiation only the Kd2 of this site was significantly decreased (13.1%, $p<0.05$).

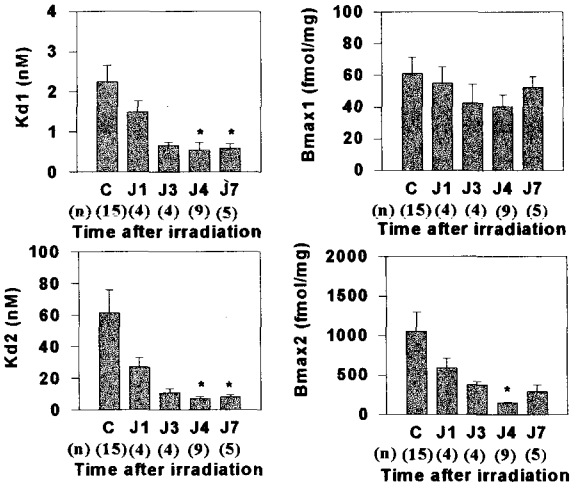

Figure.1: Characterisation of 2 muscarinic binding sites

CONCLUSION

The present data show that gamma irradiation markedly attenuates both apical (sucrase) and basolateral (Na^+/K^+-ATPase) enzyme activities. This may reflect a decrease of number and size of villosities linked to mucosal denudation (7). Such changes may explain decreased nutrient absorption following irradiation.
The modification of muscarinic receptor characteristics, in particular the effect on the high affinity binding site is in agreement with an increase of sensitivity of the small intestine to cholinergic regulation. Thus irradiation may affect regulatory processes of water and electrolyte transport. The effect on the second binding site is more difficult to interpret in terms of increased sensitivity. More experiments are required to see whether changes in both sites are also related to increased fluid and electrolyte transport after irradiation. To this end further experiments are in progress in order to test the functional capacity of muscarinic receptors using isolated intestine placed in Ussing chambers.

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