Ionizing radiation stimulates muscarinic regulation of rat intestinal mucosal function

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Lebrun, F., A. Francois, M. Vergnet, L. Lebaron-Jacobs, P. Gourmelon, and N. M. Griffiths. Ionizing radiation stimulates muscarinic regulation of rat intestinal mucosal function. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1333–G1340, 1998.—The aim of this study was to determine whether ionizing radiation modiﬁes muscarinic regulation of intestinal mucosal function. Rats exposed to total body 8-Gy γ-irradiation or sham irradiated were studied up to 21 days after irradiation. Basal and carbachol-stimulated short-circuit current (Isc) and transepithelial conductance (Gt) of stripped ileum were determined in Ussing chambers. Muscarinic receptor characteristics using the muscarinic antagonist [3H]quinuclidinyl benzilate and three unlabeled antagonists were measured in small intestinal plasma membranes together with two marker enzyme activities (sucrase, Na-K-ATPase). Enzyme activities were decreased 4 days after irradiation (day 4). Basal electrical parameters were unchanged. Maximal carbachol-induced changes in Isc and Gt were increased at day 4 (maximal ∆Isc = 195.8 ± 14.7 µA/cm², n = 19, vs. 115.4 ± 8.2 µA/cm², n = 63, for control rats) and unchanged at day 7. Dissociation constant was decreased at day 4 (0.73 ± 0.29 nM, n = 10, vs. 2.14 ± 0.39 nM, n = 13, for control rats) but unchanged at day 7, without change in binding site number. Thus total body irradiation induces a temporary stimulation of cholinergic regulation of mucosal intestinal function that may result in radiation-induced diarrhea.

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The occurrence of diarrhea may also be ascribed to radiation-induced modifications of the different systems that regulate small intestinal functions (4). The effects of ionizing radiation on some of these regulatory systems have been partly investigated during recent years. In particular, ionizing radiation has been reported to modify the blood and tissue levels of some gastrointestinal regulatory peptides, such as neurotensin and gastrin-releasing peptide (14, 22, 23), that are known to modulate intestinal blood flow, motility, and electrolyte transport. On the other hand, it can be hypothesized that a dysregulation of the autonomic nervous system may participate in the development of diarrhea induced by exposure to ionizing radiation, as has been suggested for diarrhea associated with inflammatory bowel diseases (38). In agreement with this hypothesis, the levels or effects of several neuromodulators, such as substance P and vasoactive intestinal peptide (VIP), have been reported to be modiﬁed by ionizing radiation (9, 13). Furthermore, exposure to ionizing radiation also results in altered responses to either neurally evoked electrolyte transport or to exogenously added prostaglandin E2 (10, 16, 26). Finally, Otterson et al. (30) have suggested that the abnormal contractile patterns in canine small intestine observed after irradiation may be related to impaired neural regulation or to abnormal release of gut neuropeptides.
Thus some regulatory systems have been explored, but surprisingly the effect of ionizing radiation on the classical cholinergic pathway of regulation of intestinal transport function has not been studied. Nevertheless, some indirect or direct arguments suggest that modulation of cholinergic regulation might participate in radiation-induced dysfunctions and that ionizing radiation may modulate the direct action of ACh on the enterocyte. An indirect argument was provided by experiments indicating that ionizing radiation modified levels of acetylcholinesterase (AChE), the enzyme degrading ACh (5, 11, 30). On the other hand, a direct argument was provided by the experiments of Krantis et al. (21), who reported that the contractile responses to direct smooth muscle stimulation with the muscarinic agonist carbachol was significantly increased in the duodenum and colon but not in the jejunum of the guinea pig after γ-irradiation.

Thus the aims of this study were to examine to what extent cholinergic regulation of intestinal fluid and electrolyte transport in the rat small intestine is modified by total body γ-irradiation. First, this effect was assessed in vitro in the isolated rat ileum by determination of carbachol-stimulated Isc and epithelium conductance (Gt) responses in Ussing chambers. Second, the determination of mucosal muscarinic receptor characteristics was performed using a radiolabeled muscarinic antagonist, [3H]quinuclidinyl benzilate (QNB). Finally, different muscarinic antagonists were used for the determination of receptor subtypes present in small intestinal mucosa after irradiation.

MATERIALS AND METHODS

Treatment of Animals

Male Wistar rats (Laboratoire CER Janvier) weighing between 250 and 300 g were used in all experiments, allowed food and water ad libitum, and maintained in a constant light and dark environment (12:12-h light-dark cycle).

Irradiation protocol. Conscious rats were placed in Plexiglas tubes and exposed to total body irradiation. Rats received 8-Gy γ-irradiation (60Co source), at a dose rate of 1 Gy/min. Control rats were sham irradiated during the same period. Experimental procedures were performed from 1 to 21 days postirradiation. Intestinal samples were removed under anesthesia (pentobarbital sodium, 60 mg/kg), and then animals were euthanized with an overdose of anesthetic. All experiments were conducted according to the French regulations for animal experimentation (Ministry of Agriculture, Décret no. 87–848, 19 October 1987).

Histology. Histological control of the mucosal structure of samples of jejenum and ileum was performed on another group of rats. Samples were fixed in formaldehyde and embedded in paraffin, and sections were stained with hematoxylin-eosin. Samples were observed for general morphology and organization of the villi, for the mucus state, and for the presence of inflammatory features.

Membrane Preparation

The whole ileum and jejunum were removed and rinsed with 0.9% NaCl, and all procedures were carried out on ice. The mucosal layer was scraped from the underlying muscle layers using a glass slide. For membrane preparation, tissue was homogenized in 10 volumes of sucrose buffer (250 mM sucrose, 2 mM Tris, pH = 7.4) containing the protease inhibitor phenylmethylsulfonyl fluoride (PMSEF; 0.1 mM) and centrifuged at 2,500 g for 15 min and then at 20,000 g for 20 min at 4°C. The resulting supernatant was discarded, and the pellet was resuspended in 1 ml of sucrose buffer without PMSEF, quickly frozen in liquid nitrogen, and stored at −80°C until being used for radioligand binding studies and determination of enzyme activities.

Enzyme Activities

Sucrase activity was determined as described by Mahmood and Alvarado (27), by measurement of glucose formation in the presence of glucose oxidase and peroxidase. Na+-K+-ATPase activity was estimated with the use of a ouabain-sensitive, K+-stimulated p-nitrophenyl phosphatase assay (29). Results were expressed per milligram protein, estimated using the dye-binding method of Bradford (2) with bovine serum albumin as standard.

Electrolyte Transport Studies

Segments from distal ileum and proximal jejunum were removed and rinsed with 0.9% NaCl. The segments were stripped of external muscle layer, mounted in Ussing chambers, and bathed with warmed, oxygenated (95% O2-5% CO2) Hanks’ buffer (pH 7.4) containing (in mM) 127 NaCl, 5 KCl, 0.8 MgSO4, 0.33 Na2HPO4, 0.44 KH2PO4, 1 MgCl2, 4 NaHCO3, 1 CaCl2, 5 d-glucose, 10 Na acetate, and 20 HEPES. Two samples of tissue were tested for each rat. The Isc was monitored permanently, under basal or stimulated conditions. In parallel, Gt was calculated using Ohm’s law from values of the current induced when a transepithelial potential difference (PD) of 2 mV was applied. Basal Isc, Gt, and PDt were determined after 5 min of stabilization. Tissues were then subsequently stimulated by increasing concentrations of carbachol (10–7–10–4 M) added to the serosal side of the tissue. Between each concentration of agonist, the tissue was rinsed and allowed to return to basal level. The change in Isc (ΔIsc) was determined as the difference between basal and stimulated conditions for each concentration and used for calculation of dose-response curves. The change in Gt (ΔGt) was determined as the difference in calculated Gt between basal and stimulated conditions. Both results from Isc and Gt were also expressed as percent of maximal response for each sample to allow an estimation of EC50 values (concentration of carbachol necessary to induce half-maximal response).

Radioligand Binding Studies

About 200 µg of the membrane preparation were incubated with increasing concentrations of the nonselective muscarinic antagonist [3H]QNB (specific activity 49 Ci/mmol), ranging from 50 pM to 2 nM in PBS containing (in mM) 137 NaCl, 2.7 KCl, 0.5 MgCl2, 8 Na2HPO4, 1.5 KH2PO4, 1 CaCl2, pH = 7.2, for 75 min at 30°C. For each concentration of [3H]QNB, the determinations were performed in triplicate and nonspecific binding was determined by the addition of 50 µM atropine in the incubation buffer. Nonspecific binding represented less than 10% of the total binding at concentrations of [3H]QNB near the half-maximal concentration for saturation (dissociation constant; Kd). Bound and free ligand were separated on GF/B Whatman paper filters (preincubated overnight in 0.6% polyethyleneimine), using a rapid vacuum filtration system and rinsing three times with 3 ml 10 mM cold Tris solution, pH 7.0. The experiments were performed 1, 3, 4, and 7 days after irradiation on either irradiated or sham-irradiated rats. The radioactivity was counted in a Packard liquid scintilla-
ion counter. Analysis of specific binding data was by Scatchard transformation, with the determination of values of $K_d$ and maximal binding capacity ($B_{max}$).

Antagonist Displacement Binding Studies

The effect of muscarinic antagonists on [3H]QNB binding were tested only 4 days after irradiation on sham-irradiated or irradiated rats. Membrane preparation was performed, pooling four rats for each experiment, and four experiments were performed for control and irradiated conditions. The labeled antagonist, [3H]QNB (2 nM), and increasing concentrations of three unlabeled muscarinic antagonists were used: atropine (0–5 × 10^{-3} M), pirenzepine (0–10 M), and methoctramine (0–5 × 10^{-3} M). IC50 values (concentration of antagonist necessary to induce half-maximal inhibition of [3H]QNB binding) were determined for each antagonist and for each membrane preparation.

Chemicals

Methoctramine was obtained from Research Biochemicals International, Natick, MA. Carbamylcholine chloride (carbachol), atropine, pirenzepine, and all other enzyme substrates and salts were from Sigma Chemical, Poole, UK. [3H]QNB (37 TBq/mmol) was from Amersham International, Little Chalfont, UK.

Statistical Analysis

Results are expressed as means ± SE. A one-way ANOVA was used to test populations of control rats for enzyme activities and basal electrical parameters. A one-way ANOVA Dunn’s test was used for receptor binding characteristics. Mann-Whitney’s rank sum test was applied to carbachol-stimulated increases in $I_{sc}$ and $G_i$. An unpaired t-test was used for inhibitory constant of muscarinic antagonists. Significance was set at $P < 0.05$.

RESULTS

For all experiments (determination of $I_{sc}$, enzyme activities, and receptor characteristics), no significant difference was observed between the control groups of rats tested for the different time after sham irradiation. Consequently, all results for control animals were pooled. Histological examination performed on control and irradiated rats (n = 10) showed no major modification of the mucosal structure.

Determination of Enzyme Activities

Sucrase is an enzyme associated with the apical membrane and is primarily located on the top of the villi. The results presented in Table 1 show that sucrase activity was greatly decreased to 17% of control values 4 days after irradiation ($P < 0.05$). Nine and 21 days after irradiation, the sucrose activity returned to control values (NS). In parallel, the activity of the basolateral enzyme Na+-K+-ATPase was determined. As shown in Table 1, irradiation modified Na+-K+-ATPase activity with a time-dependent pattern similar to the pattern observed for sucrose. Na+-K+-ATPase activity fell to 40% of control levels 4 days after irradiation ($P < 0.05$) and returned to control values at day 9 (NS). At day 21, a second decrease in activity of 72% was observed.

<table>
<thead>
<tr>
<th>Day</th>
<th>Sucrese Activity U/mg protein</th>
<th>Na+-K+-ATPase Activity U/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.26 ± 0.12</td>
<td>0.98 ± 0.06</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.22 ± 0.02*</td>
<td>0.39 ± 0.06*</td>
</tr>
<tr>
<td>Day 9</td>
<td>0.101 ± 0.08 (NS)</td>
<td>0.73 ± 0.09 (NS)</td>
</tr>
<tr>
<td>Day 21</td>
<td>0.79 ± 0.09 (NS)</td>
<td>0.27 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. Sucrase and Na+-K+-ATPase activities were determined on membrane preparations from mixed ileal and jejunal mucosa up to 21 days after irradiation. Experiments were performed on 4–6 rats for irradiated groups and on 9 rats for control group. Statistical difference between pooled control group and irradiated groups (*$P < 0.05$) was assessed by 1-way ANOVA. NS, not significant.

Functionality of Muscarinic Receptors: Ussing Chamber Studies

Basal values of electrical parameters. The mean values of $I_{sc}$ and $G_i$ were determined in basal conditions in ileum samples, and values obtained for control rats and irradiated rats 4 and 7 days after irradiation are reported in Table 2. The basal $I_{sc}$ value (103.1 ± 5.7 µA/cm², 63 samples for control group) was unchanged by the 8-Gy irradiation whatever the time of experiment. Similar results were obtained for basal $G_i$ (83.0 ± 3.8 mS/cm² for control group). In parallel, the basal PD$_{G_i}$ was determined. Irradiation induced no change in PD$_{G_i}$ whatever the time after irradiation (∆3.66 ± 0.18 mV for control group vs. −3.22 ± 0.34 mV for irradiated group at day 4).

Carbachol-induced increase in $I_{sc}$. Addition of carbachol to the serosal side of the ileal tissue induced a slow increase in $I_{sc}$ that reached a plateau in 4–6 min, depending on the concentration used. The increase was maintained as long as the agonist was applied. When the tissue was rinsed, the $I_{sc}$ returned to basal levels in Table 2.

| Day | $I_{sc}$ basal, µA/cm² | ∆$I_{sc}$ max, µA/cm² | ∆$I_{sc}$ EC50, µM | $G_i$ basal, mS/cm² | ∆$G_i$ max, mS/cm² | $G_i$ EC50, µM |
|-----|------------------------|------------------------|-------------------|---------------------|-------------------|_cate=0.0005      |
|     | 103.1 ± 5.7            | 115.4 ± 8.2            | 3.1               | 83.0 ± 3.8          | 57.9 ± 3.7        | 3.0               |
| 4   | 79.7 ± 8.2             | 195.8 ± 14.7*          | 3.2               | 64.7 ± 5.3          | 101.1 ± 8.1*      | 5.0               |
| 7   | 99.4 ± 7.4             | 96.2 ± 11.4*           | 4.0               | 79.9 ± 4.5          | 53.3 ± 6.4        | 3.8               |

Results from experiments performed on ileum from control rats (63 samples) and irradiated rats 4 (23 samples) and 7 (19 samples) days after irradiation are expressed as means ± SE. In dose-response curves shown in Fig. 1, the maximal (max) change in short-circuit current (∆$I_{sc}$, difference between baseline and carbachol-stimulated conditions) and change in transepithelial conductance (∆$G_i$, difference in calculated $G_i$ between basal and carbachol-stimulated conditions) were always obtained for 5 × 10^{-3} M carbachol. These maximal ∆$I_{sc}$ and maximal ∆$G_i$ values are reported in this table. Concentrations of carbachol necessary to induce half-maximal responses (EC50 values) were estimated from curves representing increases in $I_{sc}$ and $G_i$ as percent of maximal increase (curves not shown). Statistical differences between pooled control group and irradiated groups were assessed using Mann-Whitney’s rank sum test (*$P < 0.05$).
6–10 min. The dose-response curves obtained for control (63 samples) and irradiated rats at day 4 (23 samples) and day 7 (19 samples) are reported in Fig. 1. The values of maximal ΔIsc and of EC50 (estimated from curves representing increases in Isc as percent of maximal increase) are reported in Table 2. For all groups the maximal increase in Isc was obtained with a dose of 5 × 10^{-5} M of carbachol. Four days after irradiation, the amplitude of the maximal carbachol-induced increase in Isc was more important than for control conditions (maximal ΔIsc = 115.4 ± 8.2 μA/cm² for control vs. 195.8 ± 14.7 μA/cm² for irradiated rats at day 4). On the other hand, the estimated EC50 value was unchanged (see Table 2). Seven days after irradiation, the dose-response curve was again similar to control values, and no change either in maximal response or in EC50 value was observed. Similarly, no effect of irradiation on either ΔIsc or EC50 was observed at 1 and 14 days after irradiation (results not shown).

In pieces of jejunum the ΔIsc was also measured. The maximal ΔIsc, which was also obtained with 5 × 10^{-5} M carbachol, was unchanged 1 day after irradiation (92.1 ± 14.9 μA/cm², n = 9) but was increased 4 days after irradiation (114.8 ± 9.9 μA/cm², n = 15) as compared with control values (80.7 ± 11.2 μA/cm², n = 11).

Carbachol-induced increase in Gt. For ileum samples similar dose-response curves were obtained for control and Irradiated (19 samples) are reported in Fig. 1. The values of maximal Gt and of EC50 (estimated from curves representing increases in Gt as percent of maximal increase) are reported in Table 2. For all groups the maximal increase in Gt was obtained with a dose of 5 × 10^{-5} M of carbachol. Four days after irradiation, the amplitude of the maximal carbachol-induced increase in Gt was more important than for control conditions (maximal ΔGt = 0.39 nM for control vs. 0.73 ± 0.29 nM for irradiated rats at day 4). On the other hand, the estimated EC50 value was unchanged (see Table 2). Seven days after irradiation, the dose-response curve was again similar to control values, and no change either in maximal response or in EC50 value was observed. Similarly, no effect of irradiation on either ΔGt or EC50 was observed at 1 and 14 days after irradiation (results not shown).

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A change in EC50 but an important change in ΔGt 4 days after irradiation (57.9 ± 3.7 mS/cm² for control vs. 101.1 ± 8.1 for irradiated rats), with a return to control values 7 days after irradiation.

Figure 2 shows that [3H]QNB binding is reduced in a dose-dependent manner by increasing concentrations of the three muscarinic antagonists, atropine, methoc-
tramine, and pirenzepine, in sham-irradiated (Fig. 3A) and 8-Gy-irradiated rats (Fig. 3B) 4 days after irradiation. Data points of the displacement curves represent the mean of four experiments for each compound ± SE. IC50 values were estimated for each experiment and are reported in text.

**DISCUSSION**

In agreement with other studies on the rat or rabbit ileum (15, 26), no change was seen in ileal basal electrical parameters whatever the time after irradiation. MacNaughton et al. (26) reported that in rat ileum after a 10-Gy γ-irradiation basal Isc was not significantly different 2 h or 1 or 2 days after irradiation, whereas in ferret jejunum after a 5-Gy γ-irradiation basal Isc was decreased at 2 h and increased at 2 days after irradiation (25). In their conditions, G1 was not modified on the rat ileum at day 1. Furthermore, Gunter-Smith (15) observed in the rabbit no change in either distal ileal Isc or G1 during 4 days after a 5-Gy irradiation. However, an increase in basal Isc was observed from 1 day after irradiation with higher doses (7.5 and 10 Gy). In our conditions, the absence of change in basal parameters suggests no major disturbance of the integrity of the epithelial barrier. This is in agreement with our histological analysis using light microscopy, which revealed no marked structural changes, unlike what was previously observed in rat ileum at 7 days after a 5-Gy irradiation following electron microscopic analysis of the structure (31).

In contrast to the absence of change in basal parameters, maximal carbachol-stimulated responses of both Isc and G1 were increased 4 days after irradiation and returned to basal level 7 days after irradiation. No change in EC50 was observed in our conditions. These results suggest a greater capacity of ileum to respond to cholinergic stimulation 4 days after irradiation. This increased capacity of response was also observed in the jejunum, which suggests that irradiation affects in the same way the different parts of the small intestine. Our observations are in agreement with those of Harari et al. (18), who reported an increase in the effect of stimulation following a single dose of carbachol (10−4 M) at 5 days after a 7-Gy abdominal γ-irradiation. It should be noted that the timing of the effect of irradiation we observed is in agreement with other transport experiments performed on rat small intestine concerning D-glucose and water transport (1). Furthermore, our results are in agreement with experiments performed by Young and Levin (42), who reported that, in a rat model, progressive starvation for up to 3 days induced no change in either basal Isc or carbachol-induced increase in Isc in ileum 1 day after the induction of starvation. However, both basal and carbachol-stimulated Isc were increased 3 days after induction of starvation. Food intake is reduced by irradiation, which suggests that this element is a factor that may complicate the interpretation of ionizing radiation effects.

**Table 3.** Effect of an 8-Gy γ-irradiation on muscarinic receptor characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 13)</th>
<th>Day 4 (n = 10)</th>
<th>Day 7 (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kd, nM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>2.14 ± 0.39</td>
<td>0.73 ± 0.29*</td>
<td>0.78 ± 0.09</td>
</tr>
<tr>
<td>Methoctramine</td>
<td>66.0 ± 11.0</td>
<td>42.7 ± 6.6</td>
<td>69.3 ± 12.6</td>
</tr>
<tr>
<td>Pirenzipine</td>
<td>175.0 ± 36.0</td>
<td>50.0 ± 12.0*</td>
<td></td>
</tr>
<tr>
<td>Bmax, fmol/mg protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>1.21 ± 0.39</td>
<td>0.47 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Methoctramine</td>
<td>12.7 ± 1.0</td>
<td>17.5 ± 1.4*</td>
<td></td>
</tr>
<tr>
<td>Pirenzipine</td>
<td>12.7 ± 1.0</td>
<td>17.5 ± 1.4*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Receptor characteristics determined from binding experiments performed 4 and 7 days after irradiation and IC50 values obtained from antagonist displacement binding studies performed 4 days after irradiation are reported in this table. Kd, dissociation constant; Bmax, maximal binding capacity. Statistical differences between pooled control group and irradiated groups were assessed using 1-way ANOVA Dunn’s test for receptor characteristics and an unpaired t-test for IC50 values (*P < 0.05).
In our experimental conditions, the pieces of ileum placed in the Ussing chamber still contain the submucosal plexus, which may suggest that when added to the serosal side of the chamber the carbachol may act via a stimulation of some intrinsic fibers of the enteric nervous system. MacNaughton et al. (26) have tested the responsiveness of rat ileum to electrical field stimulation from 2 h to 2 days after a total body irradiation (10 Gy, $\gamma$). In these experiments they observed a decrease in the responsiveness to electrical field stimulation as soon as 1 day after irradiation. The fact that ionizing radiation modifies carbachol-induced responses differently compared with electrical field stimulation-induced responses suggests that ionizing radiation may affect cholinergic regulation of intestinal secretory responses not only at the neural level but also at the level of the enterocyte.

Many factors may contribute to changes in carbachol responsiveness of enterocyte, including alteration in 1) the concentration of drug that reaches the receptor, 2) the efficiency of binding of the drug to the receptor, 3) the number of receptor sites, and 4) the efficiency of coupling of receptors to effector mechanisms. In fact, in our in vitro studies the synthetic agonist used (carbachol) is not degraded by AChE, which suggests that the change in response observed 4 days after irradiation may be associated with either perturbation at the receptor level or at the intracellular transductional level rather than with a change in agonist concentration reaching the receptors.

Modification of receptor characteristics by ionizing radiation has already been reported in the gut for substance P, neurotensin, and VIP (9, 13, 23). Our data show that 4 days after irradiation, characteristics of muscarinic receptors of the small intestine are modified, with a decrease of $K_d$ without a change in the number of binding sites. These observations in addition to the increased intensity of change in $I_{sc}$ induced by carbachol suggest that after irradiation the small intestine is more sensitive to muscarinic regulation. In the control rats the displacement curves indicate an homogeneous population of muscarinic receptor sites. The affinity pattern of the antagonists is consistent with the presence of $M_2$ muscarinic receptors because the $IC_{50}$ for methoctramine is 10 times smaller than the $IC_{50}$ for pirenzepine. Our experiments show that after irradiation the sensitivity for the $M_2$ muscarinic antagonist (methoctramine) is decreased (increased $IC_{50}$), whereas the sensitivity for the $M_1$ muscarinic antagonist (pirenzepine) is increased (decreased $IC_{50}$) and the sensitivity for the nonselective muscarinic antagonist (atropine) is unchanged. These results show that irradiation modifies the affinity of muscarinic receptors for agonists or antagonists differently depending on the compound used, which may be due to a change in structure or access to the different binding sites.

In fact different processes may take part in modification of muscarinic receptor characteristics. A first hypothesis concerns a change in agonist level, which may induce a feedback regulation of receptor characteristics. It is conceivable that irradiation may modify the level of ACh. Indeed, irradiation has been reported to be associated with release of reactive oxygen species (ROS), interleukin-1β, and prostanoids, which have been shown to modulate the level of intestinal parasympathetic neurotransmitter liberation (12, 28, 34) and thus may lead to a decreased amount of ACh in irradiated tissue. In this study we did not measure the level of AChE, the enzyme that degrades ACh, in intestinal tissue. However, several studies that have addressed this subject indicate that ionizing radiation modifies levels of AChE, either decreasing or increasing it depending on the irradiation procedure (total body or abdominal irradiation) and on the tissue studied (5, 11, 30). In particular, whole body irradiation was reported to induce a decrease in ileal and jejunal AChE content (5, 11). These data are consistent with our observation of increased responsiveness to carbachol.

A second hypothesis deals with a possible modification of receptor environment. Ionizing radiation directly or via the production of potent ROS may damage constituents of the cell membrane such as proteins or lipids. Such modifications were reported by Keesan et al. (20) and are in agreement with the attenuation we observed of both apical (sucrase) and basolateral (Na$^+$-K$^+$-ATPase) enzyme activities. Thus ionizing radiation may have a direct effect on the molecular structure of muscarinic receptors. On the other hand, modification of protein and lipid composition can lead to an increase in membrane fluidity and a modification of the receptor environment. In this new environment, the tridimensional structure of the receptor and subsequent binding site conformation may change and so favor or disfavor agonist access to sites. This may lead to modification of binding affinities, as we have observed for muscarinic receptors. In particular, Hulme et al. (19) reported that the affinity of muscarinic receptors was differently modified by solubilization with digitonin depending on the muscarinic type considered. Indeed, the affinity of $M_2$ type receptors was altered, whereas the affinity of $M_3$ type receptors was quite unchanged.

We did not investigate the last hypothesis concerning a possible alteration by ionizing radiation of the signal transduction process associated with muscarinic receptors. Such an alteration has been observed in experiments showing that whole body irradiation of rats with $^{56}$Fe may lead to a deficit in striatal muscarinic cholinergic receptor-G protein coupling, reflected by a decrease in GTPase activity (36). Furthermore, ionizing radiation can modulate numerous other elements participating in intracellular signaling, such as intracellular calcium (17, 35), cAMP (13), and inositol trisphosphate receptors (39). Further experiments are required to determine the relative importance of modification of muscarinic transduction system in intestinal tissue.

In conclusion, in this study we observed that total body irradiation induces an upregulation of muscarinic regulation of mucosal fluid and electrolyte transport function in rat small intestine. Our results, together with those of Krantis et al. (21), show that both intestinal motility and electrolyte transport regulated by the cholinergic parasympathetic system can be
modified by ionizing radiation, which suggests that this system may be implicated in the development of radiation-induced diarrhea.

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