Reports

Synergistic Effect of 5-Bromodeoxyuridine and Gamma Rays on Chromosomes

Abstract. *The combined effect of 5-bromodeoxyuridine and gamma rays on chromosomes in cells from the root tips of* Zebrina pendula *is synergistic. The enhanced, combined effects per cell of aberrations, bridges, and acentric fragments were, respectively, 2, 2.4, and 1.9 times the sum of the separate effects.*

Greer and Zamenhof, and Greer (*1*) first reported that *Escherichia coli* became more sensitive to ultraviolet light after incorporation of the thymine analogue, 5-bromouracil. When the thymidine analogue, 5-bromodeoxyuridine (5-bromouracil deoxyriboside), is incorporated into cellular DNA the radiosensitivity of the cells increases. A partial substitution of the analogue for thymidine in the DNA of human cells in culture has led to an elevated sensitivity to ultraviolet light, x-rays, or β-rays obtained from P^{32} decay (*2*). Other evidence (*3*, *4*) of radiosensitization of *E. coli* by this analogue has been presented. The transforming principle (*4*) and phages (*4*, *5*) became more radiosensitive after the analogue was incorporated. So far the mechanism for this radiosensitization has not been clearly understood, although it has been postulated (*6*) that the incorporation of the halogen atom into DNA presumably creates a strong electrostatic repulsion between the negatively charged halogen atom and the proximate phosphate group. As a consequence, the phosphate-ester bond becomes strained and more vulnerable to radiation.

Moreover, the analogue caused chromosomal aberrations in mammalian cells in vitro (*7*). However 5-bromouracil deoxyriboside alone did not essentially increase the yield of chromosomal aberrations in *Vicia faba* (*8*) nor did it, in combination with x-rays, increase the chromosomal radiosensitivity. It did, however, counteract the blocking of the DNA replication and repairing of chromosomal breakage by 5-fluorodeoxyuridine. Likewise, the analogue exerted no observable effect by itself on chromosomes in *Vicia faba*, but it did show a radiosensitizing effect (*9*). This is a report on the combined effects of 5-bromodeoxyuridine and γ-rays on chromosomes in *Zebrina pendula* ($2n = 24$).

Stem cuttings were rooted in tap water at about 24°C. When the newly developed roots were about 2 to 3 cm long, one-half the cuttings were treated with a solution of 5-bromodeoxyuridine (5 μg/ml). Sixteen hours later one-half of the cuttings from each of the treated and nontreated groups were washed and placed between moistened filter papers in petri dishes for irradiation. The γ-rays were generated from the fission isotope products of the uranium fuel elements which were placed in the reactor operated at a power level of 1-megawatt. The material to be irradiated was placed in the irradiation chamber at a distance from the source to receive approximately 60 r/min. The total dose was 200 r. Immediately after irradiation, the cuttings were returned to tap water for recovery. Roots from all four groups, namely control, analogue-treated, γ-irradiated, and treated-plus-irradiated were fixed in Carnoy's solution (6:3:1 formula) within 3 to 24 hours. The materials were then treated with 4 percent pectinase for 1.5 hours. For cytological examination, slides with 1.5 mm-long sections of the root tips were prepared by the acetocarmine-squash method.

In this material counting of chromosomal aberrations of different categories at mitotic anaphase would produce relatively reliable results although the counting of both bridges and fragments would lead to a spurious increase in total aberrations. There were bridges and acentric fragments in the treated cells collected even within 3 hours. Other aberrations were centric segments and rings at the poles, and spurious breaks (gaps). The results are presented in Table 1. The proportion of cells with aberrations was 10.7 percent in roots treated with 5-bromodeoxyuridine, 26.8 percent in γ-irradiated roots, and 42.4 percent in roots that were treated and irradiated. Since 5-bromodeoxyuridine alone can produce the effect on chromosomes, an increase in the effect, when combined with γ-rays, over the sum of the separate effects, may be regarded as an effect of synergism. In the case just mentioned no synergism was evident although there was a higher percentage of cells with aberrations in the root meristems treated with both agents. However, the synergistic effect was apparent with respect to the production of aberrations per cell. In separate treatments the bromodeoxyuridine induced 0.24 aberration per cell and γ-rays 0.7 aberration per cell. If the effects of these two agents on chromosomes were additive, a value approximating 0.94 (0.24 + 0.7) would have been expected for the cells treated with both agents. But instead, there were actually 1.85 aberrations per cell, about twice the sum of the separate values. As expected, there was an equal degree of synergism when the numbers of bridges and fragments were analyzed separately. For the induction of bridges the combined effect was about 2.4 times the sum of the additive effects of the chemical and γ-rays and for the induction of acentric fragments the combined effect was 1.9 times the sum of the additive effects.

Some of these same results might be otherwise explained on the basis of

Table 1. Chromosomal aberration frequencies at anaphase in root tip cells from *Zebrina pendula* treated with a solution of 5 μg of 5-bromodeoxyuridine per milliliter, or γ-rays (200 r), or both.

Cells		Alterations (No. per cell)		
No.	Aberrations (%)	Aberrations*	Bridges	Fragments
		Control		
163	0.6	0.006	0	0.006
		5-Bromodeoxyuridine		
197	10.7	.24	0.06	.16
		γ-Rays		
194	26.8	.70	.21	.44
		5-Bromodeoxyuridine and γ-rays		
33	42.4	1.85	.64	1.15

* Includes bridges, fragments, centric segments and rings, and spurious breaks.

interaction of breaks induced by the two agents. Merz *et al.* (*10*) reported that in the combination treatment the interaction of breaks produced by x-rays with those produced by the chemicals, or vice versa, increased two-hit aberrations to an extent approximately twice the sum of aberrations induced by the two agents in separate treatments. In the present study, the number of bridges in the combination treatment was 2.4 times the sum of the separate treatments. This increase might well be attributed to the interaction of breaks if the bridges are assumed to result from interchanges. But the similar increase in acentric fragments arising from the combination treatment could not be explained on the very same basis because the interaction between breaks would actually reduce the yield instead of increasing it. Therefore, it is highly doubtful that the interaction of breaks was the real or major cause for the increase in the number of bridges. Additional evidence (unpublished) in favor of the synergism interpretation comes from a limited observation on aberrations at metaphase, particularly the chromatid breaks which showed about a nine-fold increase in the combination treatment over the sum of the two separate treatments (*11*).

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References and Notes

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Electrotonic Junctions between Teleost Spinal Neurons: Electrophysiology and Ultrastructure

Abstract. *Electrotonic transmission between spinal neurons is correlated with distinctive apposition of cell processes involving membrane fusion. In the same neurons, postsynaptic potentials appear to arise at typical synaptic knobs where there is an intercellular space.*

Although it is now generally believed that nervous transmission is for the most part chemically mediated (*1*) electrical transmission has been described at several vertebrate and invertebrate junctions (*2*). The two types of transmission occur at junctions that appear morphologically different. In typical vertebrate synapses where transmission is known to be chemical, the pre- and postsynaptic neurons are separated by a space of about 200 Å (*3*). Thickenings of the apposed membranes and clusters of presynaptic vesicles and mitochondria further characterize these junctions. Close apposition of membranes to actual fusion occurs at several invertebrate junctions where transmission is electrical (*4*). In smooth and cardiac muscle, fusions of apposing membranes have been considered to be the sites of electrotonic transmission between muscle fibers (*5*).

Our studies reveal two modes of indirect excitation of spinal electromotor neurons in Mormyrid electric fish (*Gnathonemus* sp., *Marcusenius* sp.). Typical postsynaptic potentials result from stimulation of descending fibers. Furthermore, a spike directly evoked in one cell spreads to all the others. These results are illustrated in Fig. 1, where responses of pairs of neurons were recorded simultaneously. The recordings

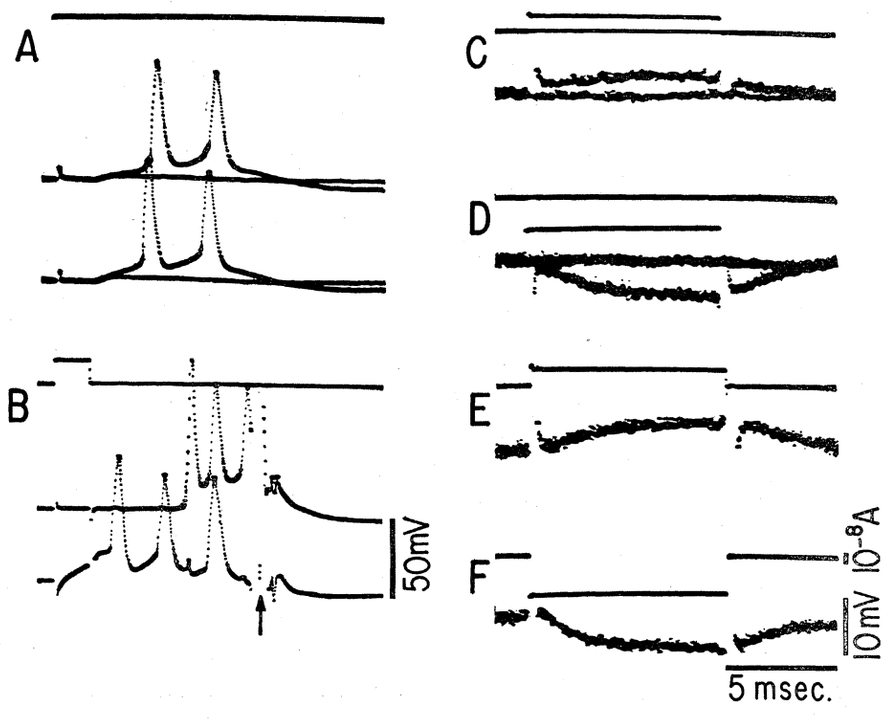

Fig. 1. Indirect excitation of electromotor neurons and electrotonic spread between them. *A*, *C–F*, from a pair of cells 0.3 mm apart; *B*, from a pair of cells 3 mm apart in a different fish. Upper trace: current applied through one recording electrode in a bridge circuit, depolarization indicated by an upward deflection. Lower traces: intracellularly recorded potentials. *A*, *B*, see text. *C*, *D*, de- and hyperpolarization of the more rostral cell of the same pair as in *A* resulted in spread of polarization to the more caudal cell (superimposed traces show the baseline). The recording from the rostral cell was omitted because of excessive bridge imbalance. *E*, *F*, when polarizing current was applied in the more caudal cell, electrotonic spread was recorded in the rostral cell. The recording from the caudal cell was omitted. The electrotonic spread was not an artifact, since the potentials were greatly reduced or absent when one or both electrodes were just extracellular.