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THE INTERRELATIONSHIPS AMONG POLY(ADP-RIBOSYL)ATION, DNA SYNTHESIS AND MAMMARY GLAND DIFFERENTIATION

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SUMMARY: Because mammary epithelium from virgin mice must undergo DNA synthesis prior to differentiation and because poly(ADP-ribosyl)ation has been linked to the cell cycle, it was hypothesized that this requirement for DNA synthesis might be related to the poly(ADP-ribosyl)ation of nuclear proteins. However, 3-methoxybenzamide, an inhibitor of poly(ADP-ribosyl)ation, stimulates α-lactalbumin accumulation even when added after DNA replication is completed. Furthermore, in parous mice this compound is still effective when DNA synthesis is blocked by cytosine arabinoside-β-D-arabinofuranoside. Therefore, poly(ADP-ribosyl)ation appears to be associated, not with DNA synthesis, but with some other event in mammary gland differentiation.

This laboratory has recently shown that differentiation in mammary gland explants is associated with a decrease in the poly(ADP-ribosyl)ation (PADPR)[1] of nuclear proteins and that differentiation can be enhanced by inhibitors of this modification (1). The time course for this change in PADPR parallels that for DNA synthesis in mammary epithelium (2); this association is not surprising, since PADPR is coupled to the cell cycle in other tissues (3, 4). Since DNA replication is an absolute prerequisite for the synthesis of milk-specific proteins in mammary epithelium from virgin mice (5), it is hypothesized that DNA synthesis may be required so that the nuclear proteins can undergo a change in PADPR; and it is the purpose of this study to investigate the potential relationship of PADPR with either DNA replication or differentiation. The former association is tested by examining the relative effectiveness of an inhibitor of this modification under various conditions affecting the cell cycle.

[1] Abbreviations: PADPR, poly(ADP-ribosyl)ation; AraC, cytosine-β-D-arabinofuranoside; 3-MBA, 3-methoxybenzamide; I, insulin; F, cortisol; P, prolactin.

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NEUTROPHILS BIOSYNTHESIZE LEUKOTOXIN, 9, 10-EPOXY-12-OCTADECENOATE

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Summary: An epoxy derivative of linoleate, 9, 10-epoxy-12-octadecenoate, was demonstrated to be biosynthesized by neutrophils from various sources such as canine and human blood, and guinea-pig peritonea. It was nominated as leukotoxin from its 'toxic' activity onto mitochondrial respiration. From the reaction mixture of leukocytes with linoleate, an isomer of leukotoxin, 12, 13-epoxy-9-octadecenoate, and a 'non-toxic' hydroxy derivative of linoleate, 9-hydroxy-12-octadecenoate, were detected. Such a cascade reaction of linoleate by leukocytes was discussed. Biosynthesis of leukotoxin by neutrophils was substantially enhanced by the presence of calcium ion and calcium-ionophore, A_{23187}. Neutrophils contained leukotoxin, *ca.* 7 f moles/cell, which was extractable by 60% ethanol, but little of the isomer.

In the previous papers, we reported the existence of leukotoxin (LX), 9, 10-epoxy-12-octadecenoate, and its isomer (LX'), 12, 13-epoxy-9-octadecenoate, in lung lavages of rat after exposure to hyperoxia and in the reaction mixture of the lung lavage leukocytes with linoleate(1) as well as its existence in human burned skin (2). 'Toxic' effect of leukotoxin on mitochondrial respiration and on smooth muscle contraction was noted (1). Recently, we have been informed from Kato et al. (3) that they found exactly the same linoleate epoxides exist in the rice plant as self defensive substances against rice blast disease caused by a fungus, *Pyricularia orgzae*, of which characteristic is antimycin-sensitive. These facts indicate that LX is a common substance among animal and plant.

Abbreviations: HPLC, high performance liquid chromatography; GC-MS, gas-chromatography/mass spectrometry; NMR, nuclear magnetic resonance; RCI, respiratory control index; St III O_2, the rate of oxygen consumption in State III respiration; PLase, phospholipase; Percoll, polyvinylpyrrolidone-coated silica gel.

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as the addition prior to DNA synthesis. This experiment also demonstrates that 3-MBA acts only during differentiation (i.e., when all three hormones are present) and reaffirms the reciprocal association between PADPR and mammary gland differentiation.

An alternate way of testing the DNA synthesis hypothesis is to determine if 3-MBA is active in the presence of an inhibitor of DNA synthesis, such as AraC. This cannot be tested in tissue from virgins, since the epithelium will not undergo differentiation when DNA synthesis is inhibited; however, such tissue can be used as a negative control (Table 2). In contrast, explants from neither midpregnant nor parous mice require DNA synthesis (10, 11) and are unaffected by AraC (Table 2). Furthermore, in the presence of AraC, tissue from parous animals still respond to 3-MBA, thereby supporting the double-incubation experiment described above. However, it is interesting to note that explants from pregnant mice do not respond to 3-MBA under any circumstances; presumably the PADPR of the nuclear proteins in this tissue has already been reduced to minimal levels earlier in pregnancy.

Although it would have been interesting to measure PADPR synthetase activity in the above experiments, this was not technically possible: whenever AraC was present in the media, enzyme activity was extremely high. This enzyme has been

TABLE 2

Effect of Inhibiting DNA Synthesis on the Actions of 3-Methoxybenzamide (3-MBA) on Mammary Gland Differentiation

	α-Lactalbumin (ng/mg wet tissue)		
Incubation	Virgin	Midpregnant	Parous
IFP	9.5 ± 1.9	103 ± 13	16.4 ± 3.0
IFP + 3-MBA	22.1 ± 2.5	96 ± 18	31.1 ± 7.1
IFP + AraC	0.9 ± 0.7	109 ± 13	17.3 ± 3.0
IFP + 3-MBA + AraC	1.2 ± 0.5	124 ± 7	31.9 ± 4.3

Mammary gland explants from virgin, pregnant or parous mice were cultured in IFP with or without 3-MBA (1 mM) and/or AraC (15 μg/ml) as indicated. After 3 d, α-lactalbumin was determined; each value is the mean ± S.E. for three separate experiments.

implicated in DNA repair and can be activated by DNA breaks (12). Since AraC inhibits DNA synthesis, it might be expected to allow the accumulation of damaged DNA, thereby activating the synthetase. Alternatively, this activation may simply represent some unexplained side-effect of AraC.

If the action of 3-MBA is associated with differentiation, rather than DNA replication, is its action restricted to early differentiation or must it be continuously present? Table 3 shows that α-lactalbumin accumulation on day 4 of culture is statistically the same ($p>0.05$) for both explants continuously exposed to 3-MBA and also for tissue exposed only during the first 2 days (a one-day exposure is inadequate to produce a sustained response). However, by the fourth day following removal of 3-MBA, α-lactalbumin content returns to the IFP-control level. These results show that 3-MBA must be continuously present to have its full effect, although the transient response at 2 days implies that the half-time of this effect is relatively long. This prolonged effect is not due to a persistent depression of PADPR synthetase activity,

TABLE 3

Effect of Different Exposure Times of 3-Methoxybenzamide (3-MBA) on Mammary Differentiation

Incubation			
First	Second	α-Lactalbumin (ng/mg wet tissue)	Poly (ADP-ribosyl)synthetase activity (pmol ribose incorp./μg epithelial DNA)
IFP (4 d)	-	24.0 ± 3.2	0.32 ± 0.05
IFP + 3-MBA (4 d)	-	55.8 ± 9.4	0.10 ± 0.02
IFP + 3-MBA (1 d)	IFP (3 d)	26.4 ± 5.6	0.33 ± 0.04
IFP + 3-MBA (2 d)	IFP (2 d)	40.4 ± 7.0	0.30 ± 0.06
IFP (6 d)	-	27.8 ± 2.2	0.38 ± 0.09
IFP + 3-MBA (6 d)	-	57.5 ± 5.8	0.12 ± 0.04
IFP + 3-MBA (2 d)	IFP (4 d)	28.8 ± 2.5	0.36 ± 0.08

Mammary gland explants from virgin mice were cultured in hormones (as indicated) with or without 3-MBA (1 mM). Each value is the mean ± S.E. for either three (upper panel) or four (lower panel) separate experiments.

since such activity returns to control values by 1 day after the removal of 3-MBA (Table 3).

All of the above data suggest that the decrease in PADPR seen during mammary gland development is not coupled to DNA synthesis, but rather it is associated with differentiation. Furthermore, the change in PADPR does not appear to occur as a single, fixed event, but must be continuously maintained.

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