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CELL CYCLE INHIBITION BY SODIUM BUTYRATE IN LEGUME ROOT MERISTEMS

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Key Word Index—*Glycine max*; *Phaseolus vulgaris*; *Vicia faba*; Leguminosae; root meristems; sodium butyrate; cell cycle.

Abstract—Previous investigations demonstrated that butyric, propionic and valeric acids reduced the mitotic index in cultured root meristems of peas (*Pisum sativum*). These compounds also inhibited DNA synthesis and the progression of cells through the cell cycle. Cultured pea roots, exposed to either 0.1 mM ^{14}C-labelled butyrate or propionate, incorporated the isotope and transported it into the nucleus. Experiments have now been performed to determine if cultured roots of other legume species such as soybean (*Glycine max*), pinto bean (*Phaseolus vulgaris*) and broad bean (*Vicia faba*) also respond to butyrate in a similar manner. After a 24-hr butyrate exposure, the mitotic index in the root meristems was greatly reduced in all three test species, even at butyrate concentrations as low as 0.1 mM. The mitotic index in broad bean roots exposed to either 1 or 5 mM butyrate was reduced as quickly as 6 hr post-exposure. When broad bean roots were exposed to 5 mM butyrate, [^{3}H]-TdR incorporation was inhibited by 8 hr, indicating a reduction in DNA synthesis. These experiments demonstrate that other legume species respond to butyrate exposure by reducing their overall mitotic index and halting progression through the cell cycle.

INTRODUCTION

Short-chain aliphatic fatty acids have a wide range of effects on both plant and animal cells. Specifically, butyric acid at millimolar concentrations inhibits DNA synthesis in both mouse and HeLa cells [1, 2]. Sodium butyrate has also been implicated in switching the specialization of committed embryonic rat liver progenitor cells [3].

A number of experiments have implicated butyrate in the control of cell proliferation in plant tissues, especially in root apical meristems. Butyrate inhibited DNA synthesis in lettuce (*Lactuca sativa*), arresting the nuclei in both the G1 and G2 phases of the cell cycle [4]. Similar effects were observed when root meristems of onion (*Allium cepa*) and pea (*Pisum sativum*) were exposed to butyrate [5, 6]. Additional butyrate effects in plants include the inhibition of gibberellic acid-induced synthesis and secretion of α-amylase [7], and the inhibition of germination in *Phacelia tanacetifolia* [8].

Previous investigations in this laboratory demonstrated that butyric, propionic and valeric acids reduced the mitotic index in cultured root meristems of peas (*Pisum sativum*). These compounds also inhibited DNA synthesis and progression through the cell cycle [9, 10]. When cultured pea roots were exposed to either 0.1 mM ^{14}C-labelled butyrate or propionate, meristem cells incorporated the isotope over time and eventually translocated it to the nucleus [11].

Experiments were conducted to demonstrate that root meristems of other legume species (soybean, pinto bean and broad bean) responded to butyrate in a similar manner to that of peas, by reducing the overall mitotic index and halting progression through the cell cycle by inhibiting DNA synthesis.

RESULTS AND DISCUSSION

Figure 1 shows the effects of various concentrations of sodium butyrate on the mitotic index of root meristem cells of soybean, pinto bean, and broad bean, respectively. All three test species responded to butyrate in a similar manner. Control mitotic indices ranged between 3.8 and 5.2% depending on the species. After a 24-hr butyrate exposure, the mitotic indices were significantly lower than those of the controls, demonstrating a dose–response relationship. Higher concentrations of butyrate showed the greatest decline in overall mitotic indices. Of the three test species, pinto beans were most sensitive to the increase in butyrate concentrations, essentially eliminating mitotic figures at 5 mM exposure.

In order to determine when the inhibitory effect of butyrate was initiated, cultured root meristems of broad bean were exposed to either 1 or 5 mM butyrate and were sampled at 2-hr intervals over a 12-hr exposure period. Control roots demonstrated a fluctuating mitotic

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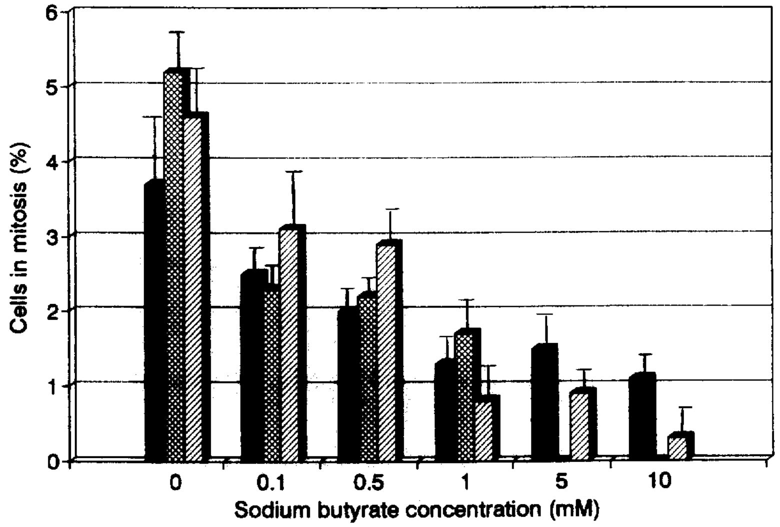

Fig. 1. Effects of various concentrations of sodium butyrate on the mitotic index of excised root meristems of various legumes (dark shading, *Glycine max*; light hatching, *Vicia faba*; cross hatching, *Phaseolus vulgaris*) grown in White's medium with sucrose for 24 hr. Mean and SE of at least three slides; 1000 nuclei were scored per slide. Each experiment was performed at least twice.

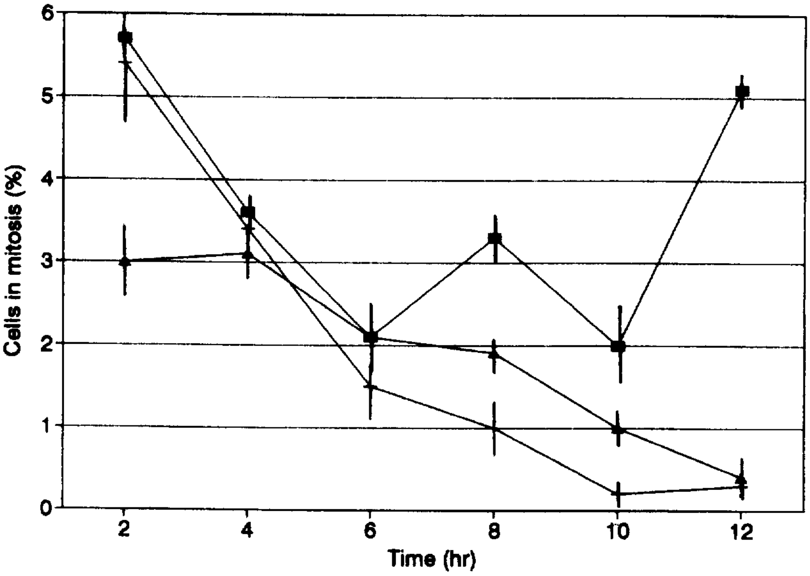

Fig. 2. Effects of 1.0 (▲) and 5.0 mM (+) butyrate on the mitotic index of excised root meristems of broad bean (*Vicia faba*) grown in White's medium with sucrose for various time periods (■, no butyrate). Seven-day-old seedlings were initially employed. Mean and SE of at least three slides; 1000 nuclei were scored per slide. Each experiment was performed at least twice.

index between 2 and 5% over the test period, whereas butyrate roots began to manifest their mitotic inhibition by 6 hr, and the mitotic indices were essentially zero by the 12-hr sample (Fig. 2). It is uncommon for cultured roots to demonstrate an initial drop in mitotic activity immediately after transfer to culture medium, however, this effect usually lasts for only 4–6 hr.

To ascertain whether DNA synthesis in other legume species was affected by butyrate, cultured roots of broad bean were exposed to [^{3}H]-TdR for a period of 12 hr and sampled at 2-hr intervals. Results show that for the first 8 hr of butyrate exposure, [^{3}H]-TdR incorporation rates were similar to controls; however by 12 hr, the rate of [^{3}H]-TdR incorporation was significantly higher in controls, suggesting that DNA synthesis was being impeded (Fig. 3).

The role of butyrate in halting mitosis and inhibiting DNA synthesis has been previously elucidated in a variety of diverse plant species such as lettuce, onion and peas [3–5]. The list of responsive species can now be

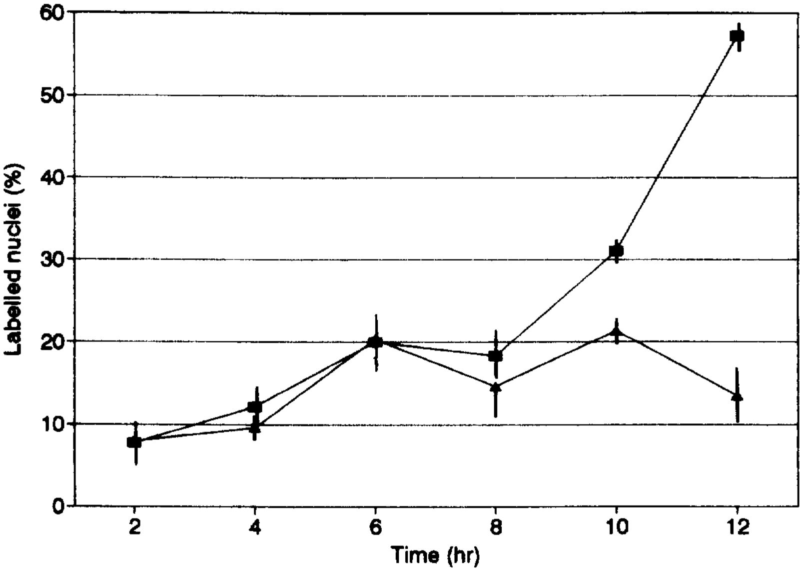

Fig. 3. Incorporation of [^{3}H]-TdR by cultured root meristem nuclei of broad bean (*Vicia faba*) grown in White's medium with sucrose for various time periods in the presence (▲) or absence (■) of 5 mM sodium butyrate. Seven-day-old seedlings were employed. Mean and SE of at least three slides; 500 nuclei were scored per slide. Each experiment was performed at least twice.

expanded to include soybeans, pinto beans and broad beans. These three species respond to butyrate in a manner similar to that of the previously tested legume species peas. Susceptibility to butyrate varied somewhat between the test species, with pinto beans being the most sensitive. Cultured root meristem nuclei of peas incorporate ^{14}C-labelled butyrate [10]. The three test species also showed mitotic inhibition and repression of DNA synthesis, suggesting that butyrate was also taken into the nucleus. However, exactly how butyrate exerts its effects in the nucleus remains unclear.

Finally, derivatives of butyrate have been known to accumulate in plant tissues following exposure to the herbicide chlorosulfuron [12, 13]. Both 2-ketobutyrate and 2-amino-*n*-butyrate accumulate in chlorosulfuron-treated roots. Chlorosulfuron at 28 mM reduced the mitotic index in pea roots to 0% after 8 hr [14]. These observations lend credence to the hypothesis that butyrate (or one of its derivatives) has the ability to act as a cell cycle regulator.

EXPERIMENTAL

Germination of seedlings. Seeds were surface-sterilized with undiluted Clorox for 10 min, washed with sterile H_2O and germinated in the dark at 23° in sterile vermiculite. Germination periods were 3, 4, and 7 days, respectively for *P. vulgaris*, *G. max*, and *V. faba*.

Culture conditions. Excised roots from the appropriate age seedlings were placed into culture flasks containing White's medium with Na butyrate at various concns.

Determination of mitotic indices. Root segments were harvested at various times after butyrate exposure. The roots were fixed in 95% EtOH:HOAc (3:1), 30 min, and transferred to 70% EtOH. The roots were stained with Feulgen reagent and the terminal 0–2 mm meristems were squashed onto slides in a drop of 45% HOAc. A cover glass was placed over the tissue, and the slide was pressed between blotting papers to spread the cells. The cells fixed by placing the slide on block of dry ice for several min, then popping off the cover slip and placing slide into 95% EtOH. Slides dehydrated through xylene and made permanent with Canada Balsam and a fresh cover glass. The slides (at least 3 per treatment) scored for mitotic index by counting the number of mitotic figures per 1000 cells and expressing this as a percentage.

[^{3}H]-*TdR labelling of meristem cells.* Seedlings of *V. faba* were continually exposed to [^{3}H]-TdR (37 MBq ml^{-1}, 248 TBq mol^{-1}) in aerated White's medium. Samples taken at various times in order to estimate the number of cells synthesizing DNA. Roots fixed, stained with Feulgen, and the terminal 0–2 mm meristem squashed on a slide and dipped in Kodak NTB-2 emulsion (Kodak Corp.). After 7 days of exposure, slides developed and prepd. for microscopy. Labelled nuclei possessed more than 5 silver grains per nucleus; nuclei with 5 grains or fewer were considered unlabelled.

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