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A Cardiotonic Steroid Bufalin-Induced Differentiation of THP-1 Cells

INVOLVEMENT OF NA^+, K^+-ATPase INHIBITION IN THE EARLY CHANGES IN PROTO-ONCOGENE EXPRESSION

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ABSTRACT. Human monocytic leukemia THP-1 cells were induced to differentiate into macrophage-like cells by treatment with cardiotonic steroid bufalin, which was previously shown to interact with the Na^+, K^+-ATPase with similar kinetics to ouabain, a specific inhibitor of the enzyme. This induction of differentiation was characterized by loss of proliferation, cell adherence, increased ability to reduce Nitro Blue tetrazolium (NBT), and increased expression of interleukin 1β (IL-1β). During this process, bufalin downregulated c-myb and c-myc expressions and induced c-fos and Egr-1 transcripts. Ouabain also caused similar changes in proto-oncogene expression and induced phenotypic markers of differentiated cells at concentrations comparable to bufalin. The 12-O-tetradecanoyl phorbol-13-acetate resistant THP-1 cell variant, which was unresponsive to this agent as to growth inhibition and proto-oncogene expression, responded to bufalin. The finding that protein kinase inhibitor H7 failed to inhibit bufalin-mediated c-fos induction further supports the theory that the signal transduction machinery caused by bufalin is separable from the phorbol ester. The cytotoxic effect of high doses of bufalin apparently disappeared in the medium where Na^+ was replaced with choline ions. Furthermore, bufalin failed to induce c-fos expression and to downregulate c-myb transcripts in the low-Na^+ medium. These findings indicate that an increased intracellular Na^+ concentration resulting from the Na^+, K^+-ATPase inhibition possibly triggers the change in proto-oncogene expression evoked by bufalin. BIOCHEM PHARMACOL 52;2: 321–329, 1996.

KEY WORDS. cell differentiation; Na^+, K^+-ATPase; bufalin; proto-oncogenes; THP-1; TPA resistance

The toad venom preparation *Ch'an Su* or *Senso* has widely been used as a cardiotonic agent. The known pharmacological action of the Chinese medicine resides in Na^+, K^+-ATPase (EC 3.6.1.3) inhibition by cardiotonic steroids, the so-called bufadienolides. We have reported that bufadienolides, as well as ouabain, which is known to be a specific inhibitor of Na^+, K^+-ATPase, are capable of inducing human leukemia cell differentiation [1–3]. Among the major bufadienolides, bufalin is the most effective inducer [3]. The effects of bufalin on leukemia cells are characterized by species-selective sensitivity (human-derived cells are more susceptible to bufalin than animal-derived cells), and by its broad spectra on human cells arrested at different stages [1–3]. These unique features of the effect of bufadienolides on leukemia cells prompted us to try to clarify the induction mechanism that would be of interest in understanding leukemia cell differentiation. We have previously shown that differentiation-inducing activities of bufadienolides correlated well with those Na^+, K^+-ATPase inhibitory abilities in human erythroleukemia K562 cells [3]. In addition, the majority of bufalin added to a medium may bind to Na^+, K^+-ATPase at the cell membrane, rather than be incorporated into cells [3]. Based on these findings, we have proposed that bufadienolide-mediated induction of cell differentiation may result from Na^+, K^+-ATPase inhibition. To confirm this hypothesis, we have attempted to determine whether bufalin-induced changes in proto-oncogene expression are mediated by enzyme inhibition. Here, we show that an accumulation of c-fos mRNA and a concomitant decrease in c-myb expression during bufalin-mediated dif-

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¶ *Abbreviations:* TPA, 12-O-tetradecanoyl phorbol-13-acetate; H7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine; XTT, 3,3′-[1[(phenylamino)carbonyl] 3,4-tetrazolium]-bis(4-methoxy-6-nitro)-benzenesulfonic acid; NBT, nitro blue tetrazolium; IL-1β, interleukin 1β; PKC, protein kinase C; EDLFs, endogenous digitalis-like factors; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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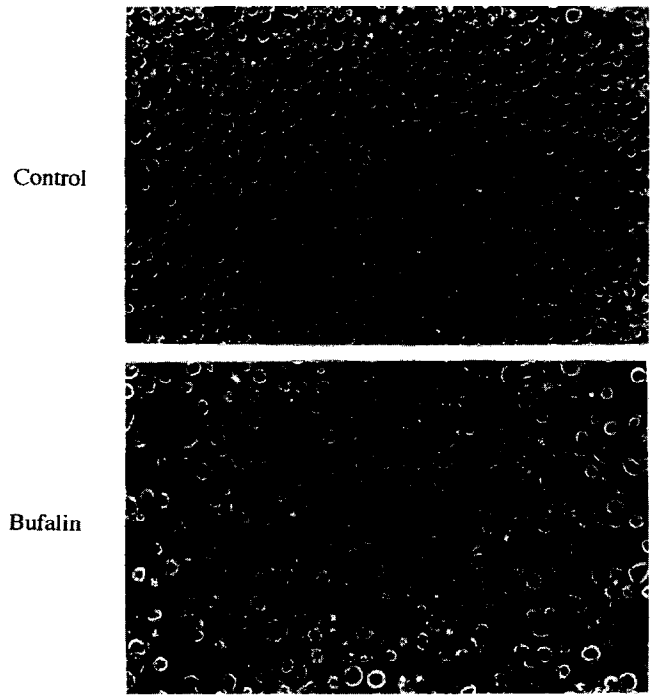

FIG. 1. Morphology of bufalin-treated THP-1 cells. Cells were incubated in the absence (control) or presence of 30 nM bufalin for 5 days. Cell morphology was examined under a light microscope (magnification, ×200).

ferentiation of human monocytic THP-1 cells are largely dependent on extracellular Na^+ concentration. Thus, it is strongly suggested that Na^+, K^+-ATPase can be a target of leukemia cell differentiation.

MATERIALS AND METHODS

Materials

Bufalin was isolated from chloroform extracts of a Chinese toad venom preparation (Senso: Shibata Pharmaceutical Co., Tokyo, Japan) by repetitive chromatography on silica gel and a LiChroprep RP-18 Lobar column (E. Merck, Darmstadt, Germany), as described previously [3]. Amiloride, TPA[11] and ouabain were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). H7 was from Seikagaku Kogyo Co. (Tokyo, Japan). XTT was purchased from Polysciences, Inc. (Washington, PA, U.S.A.).

Cell Cultures

THP-1 cells [4] were provided by the Japanese Cancer Research Resources Bank (JCRB). The cells were grown in RPMI-1640 medium (Gibco Lab., Grand Island, NY, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (FBS, ICN/Flow, Irvine, Scotland), 20 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, 0.2% sodium bicarbonate, and penicillin-streptomycin. Cells were seeded at 1×10^5 cells/mL and maintained in continuous logarithmic growth in a humidified 5% CO_2 atmosphere at 37°C. A TPA-resistant THP-1 subclone (THP-1/TPA) was established by cultivating THP-1 cells in the presence of increasing concentrations (0.1 ~ 20 nM) of TPA over a period of 5 months. The resistant cells were maintained in the medium containing 20 nM TPA and were cultivated without the phorbol for 1 week before use.

Growth Inhibition and Induction of Differentiation

Bufalin and ouabain were dissolved in absolute ethanol and redistilled water, respectively, to make 2 mM stock solutions that were appropriately diluted with Dulbecco's PBS just before addition to the cell culture. The final ethanol concentration in the incubation medium was less than 0.1%, which had no effect on cell growth. Growing cells were subcultured at 0.5×10^5 cells/mL with various concentrations of bufadienolides and were incubated for 3 days in a 12-well multidish. A viable cell number and the per-

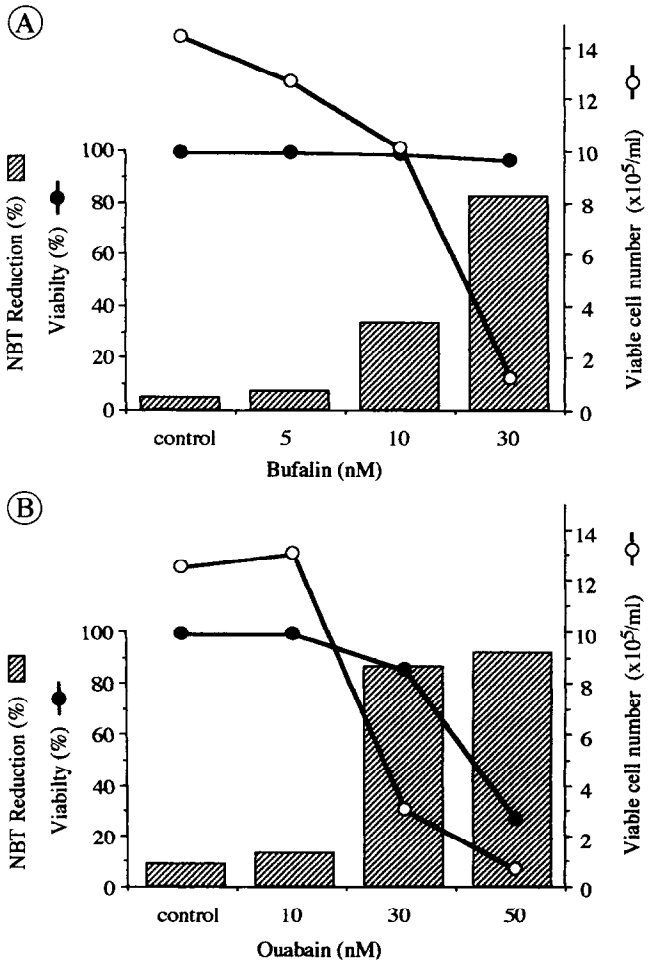

FIG. 2. Growth inhibition and induction of differentiation of THP-1 cells by bufalin and ouabain. Cells (1×10^5 cells/mL) were treated with various concentrations of bufalin (A) or ouabain (B) as indicated for 4 days. Viable cell number (open circle) and viability (closed circle) were determined by the Trypan Blue dye exclusion test. NBT reduction (column) assay was carried out in the presence of 0.1 μg/mL TPA for 30 min. The mean of 3 determinations is illustrated.

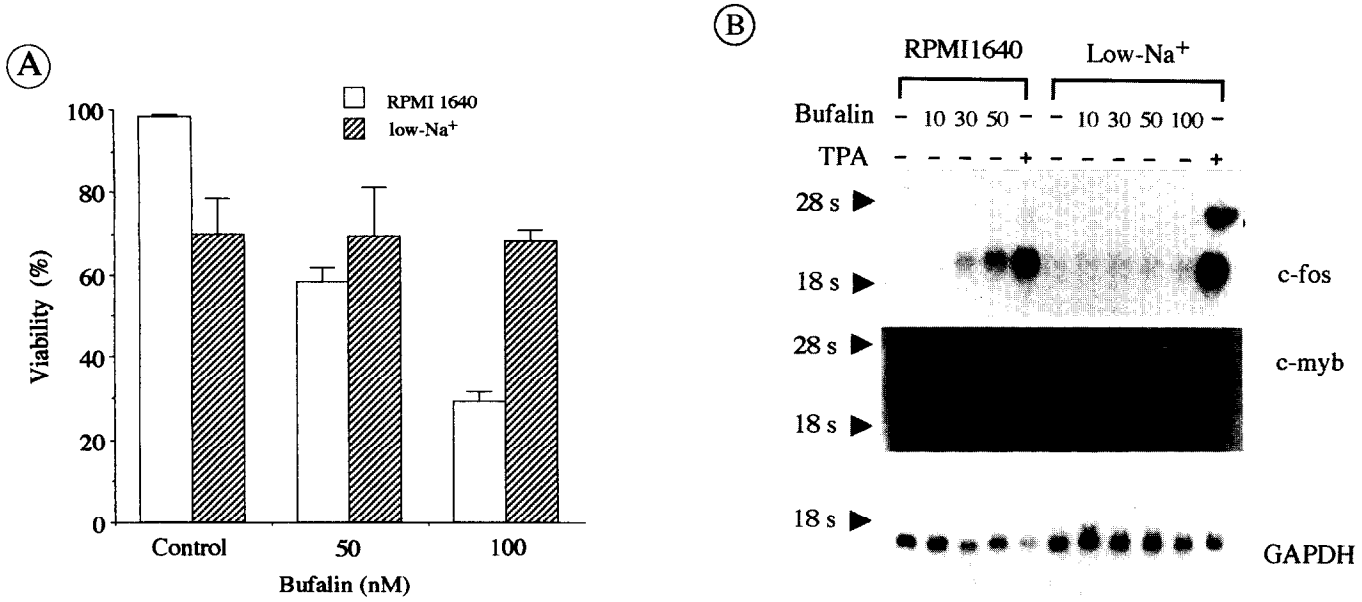

FIG. 3. (A) Cytotoxic effect of bufalin on THP-1 cells in the low-Na^+ medium. Cells (1×10^5 cells/mL) were incubated in the RPMI 1640 (open column) or low-Na^+ RPMI 1640 medium (hatched column and circle) in the absence (control) or presence of 50 or 100 nM bufalin for 48 hr. The percentage of viable cells (viability) was determined by Trypan Blue dye exclusion test. The mean ± SE of 3 determinations is illustrated. (B) Effect of bufalin on the expression of c-fos and c-myb proto-oncogenes in THP-1 cells cultivated in the low-Na^+ medium. Cells were incubated in the RPMI 1640 or the low-Na^+ RPMI 1640 medium in the presence of bufalin at the concentrations indicated for 24 hr. Cells were also treated with 10 nM TPA for 24 hr as a positive control. Expressions of c-fos and c-myb proto-oncogenes were analyzed on Northern blots as described in the legend to Fig. 4 and Experimental Procedure.

centage of viable cells (viability) were determined by the Trypan Blue exclusion test. Respiratory burst was evaluated by assessing the ability to reduce NBT dye, as described previously [1]. In some experiments, growth inhibition of the drugs was determined by the XTT formazan assay in a 96-well multidish essentially according to the method described by Scudiero *et al.* [5].

Northern Blot Analysis

Total RNA was isolated from the cells by acid guanidium thiocyanate-phenol-chloroform extraction as described by Chomczynski and Sacchi [6]. Total RNA (20 μg/lane) denatured with 50% formamide/2.2 M formaldehyde was subjected to electrophoresis in 1% agarose gel containing 2.2 M formaldehyde and transferred to a Nytran membrane (Schleicher and Schuell, Dassel, Germany). The RNA blots were hybridized in the presence of 50 mM sodium phosphate buffer, pH 6.5, 5× Denhardt's solution (1× Denhardt's, 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin), 50% formamide, 5× SSC (1× SSC, 0.15 M NaCl/0.015 M sodium citrate), and 280 μg/mL denatured salmon sperm DNA with ^{32}P-labeled probes. cDNA probes were labeled by the multiprimer DNA reaction (Megaprime DNA labeling system, Amersham, Bucks, U.K.) with [α-^{32}P]-dCTP (3000 Ci/mmol, Institute of Isotopes Co., Hungary). After overnight hybridization at 42°C, the blots were washed twice for 30 min, each time at 42°C with 2 × SSC/0.1% SDS, and then twice for 30 min, each time with 0.1 × SSC/0.1% SDS at 42°C. Relative mRNA contents were quantitated by the Fuji BAS 3000 image analyzer (Fuji Photo Film Co., Kanagawa, Japan). Filters were then exposed to X-ray film (Fuji Photo Film) at −70°C, using an intensifying screen. The probes used were the 1.7 kb EcoRI/PstI fragment of a human c-fos cDNA purified from pSPT-fos cDNA plasmid [7] (JCRB), the 2.5 kb EcoRI insert of a human c-myb cDNA purified from myb cDNA-C plasmid [8] (JCRB), the 0.85 kb EcoRI/ClaI fragment of a human c-myc cDNA purified from pSPT-myc cDNA plasmid [9] (JCRB), the 1.5 kb PvuII fragment of a human Egr-1 cDNA purified from ETR103 plasmid [10] (provided by Dr. H. Utiyama through Riken DNA Bank, Japan), the 1.1 kb PstI insert of a human IL-1β cDNA purified from IL-1 X-14 plasmid [11] (American Type Culture Collection), and the 0.5 kb insert of a rat GAPDH cDNA purified from GD5 plasmid [12] (provided by Dr. T. Sato, University of Tottori through Dr. K. Nose, Showa University).

RESULTS

Bufalin Induces THP-1 Cell Differentiation

Bufalin induces differentiation of leukemia cells of human origin arrested in different lineages [1, 2]. Among the leukemia cell lines we have tested so far (K562, HEL, KG-1, ML1, U937, HL60, and THP-1), the most striking changes in morphology after treatment with bufalin were observed in THP-1 cells (Fig. 1). THP-1 cells that had been incu-

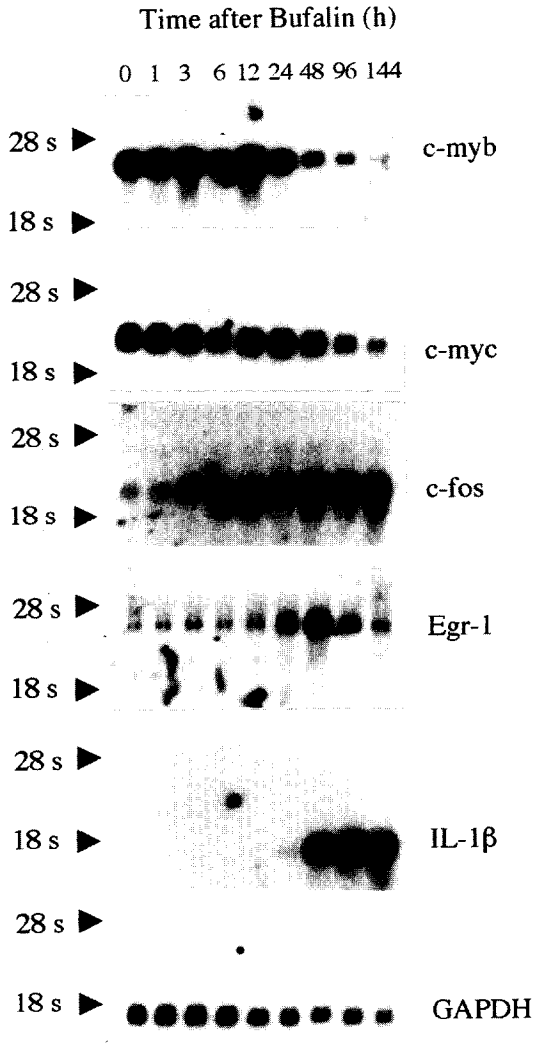

FIG. 4. Time-course of the effect of bufalin on proto-oncogene and IL-1β expression in THP-1 cells. Cells were treated with 30 nM bufalin for the times indicated. Northern blots were prepared from total RNA (20 μg/lane) and hybridized with ^{32}P-labeled probes indicated. Blots were also rehybridized with a GAPDH probe to analyze RNA loading. One representative experiment of 4 is shown.

bated for 5 days in the presence of 30 nM bufalin showed a typical macrophage-like amoeboid shape. This cell line primarily grows in suspension; however, approximately 25% of the cell population of the bufalin-treated cells tightly adhered to the tissue culture plate after 4 days (data not shown). In addition, bufalin induced growth arrest and production of superoxide anion as determined by NBT dye reduction in THP-1 cells dose-dependently (Fig. 2A). However, concentrations of more than 50 nM were cytotoxic to the cells (Fig. 3A). Expression of IL-1β mRNA, a functional marker for terminal differentiation of monocytoid cells, appeared 48 hr after treatment of the cells with 30 nM bufalin, and a significant level of the mRNA was observed 96 hr later (Fig. 4). These data indicate that bufalin induces THP-1 cells to undergo terminal differentiation into macrophage-like cells. Ouabain has been shown to induce K562 cell differentiation with lesser potency than bufalin [3]. In THP-1 cells, however, ouabain at a concentration of 30 nM effectively induced growth arrest, superoxide production (Fig. 2B), and IL-1β expression (data not shown), as observed with bufalin.

Effect of Bufalin on Proto-oncogene Expressions in THP-1 Cells

Expression levels of transcription factors that have roles in growth and differentiation of hematopoietic cells, during bufalin-induced THP-1 cell differentiation, were determined by Northern blot analyses. Constitutively expressed c-myb and c-myc mRNAs decreased continuously during bufalin-induced THP-1 cell differentiation (Fig. 4). Downregulation of c-myb and c-myc mRNAs appeared 48 hr and 96 hr, respectively, after bufalin treatment (Figs. 4 and 5A). On the other hand, c-fos mRNA, whose expression was very low in untreated THP-1 cells, increased within 3 hr of bufalin stimulation and accumulated further up to 144 hr (Fig. 4). Expression of another immediate early response gene, Egr-1, which is known to be induced during macrophage differentiation [10], was also induced; however, this was seen only after 24 hr of treatment (Fig. 4). In addition, the induction of Egr-1 mRNA was transient; it reached a peak at 48 hr and declined thereafter. On the other hand, expression of a housekeeping gene, GAPDH, was not affected by bufalin. The effect of bufalin on proto-oncogene expression was observed in a dose-dependent manner (Fig. 5A) and appeared at doses of 30 nM or higher, which effectively induced the differentiation markers in the cells (Fig. 2A). Like bufalin, ouabain also produced similar proto-oncogene expression in THP-1 cells (Fig. 5B).

Protein Kinase C is Not Involved in Bufalin-Mediated Signal Transduction

TPA-induced THP-1 cell differentiation is also associated with downregulation of c-myb and c-myc expressions and induction of c-fos and Egr-1 transcripts [13]. These effects of TPA are thought to be coupled with PKC activation. The next series of experiments, therefore, were conducted to determine whether or not PKC-mediated signal transductions are involved in the effect of bufalin on the expression of transcription factor mRNAs. We established TPA-resistant THP-1 (THP-1/TPA) cells by long-term cultivation of the cells in the presence of increasing amounts of TPA. THP-1/TPA cells that had been cultivated in a medium without TPA for 7 days showed nearly 1000-fold resistance to TPA treatment, in terms of growth inhibition, in comparison to the parental cells (Fig. 6A). TPA-induced downregulation of c-myb (Fig. 7A) and c-myc (data not shown) mRNAs observed in THP-1 cells virtually disappeared in THP-1/TPA cells. Induction of c-fos and Egr-1 mRNAs by TPA was also attenuated in the resistant cells (Fig. 7A). In addition, no morphological changes, including cell adherence, were observed in THP-1/TPA cells after treatment with TPA (data not shown).

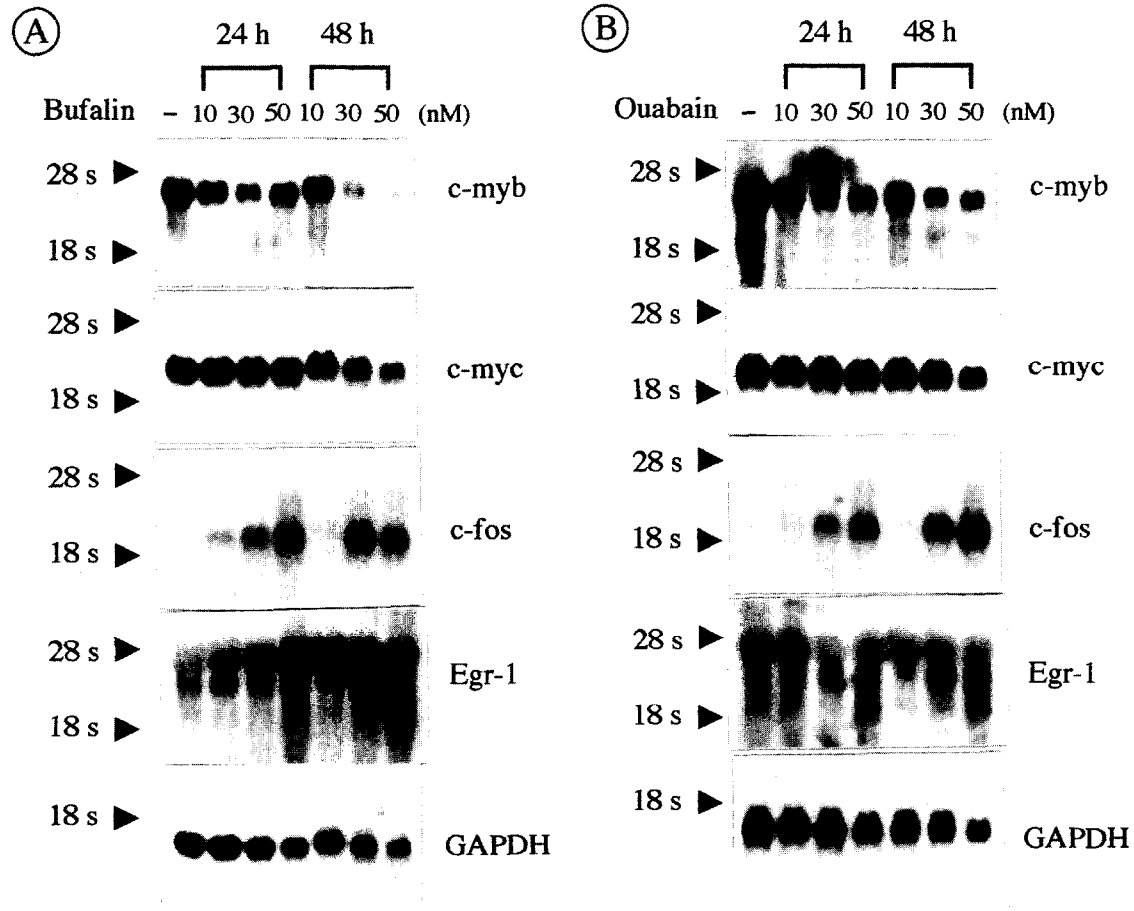

FIG. 5. Dose-related effects of bufalin and ouabain on proto-oncogene expressions in THP-1 cells. Cells were incubated with bufalin (A) or ouabain (B) at concentrations indicated. After 24 or 48 hr, total RNA was isolated from the cells and analyzed on Northern blots, as described in the legend to Fig. 4 and Experimental Procedure.

These results demonstrate that THP-1/TPA cells are defective in TPA-induced signaling events. Bufalin inhibited growth of THP-1/TPA cells at concentrations comparable to those effective against THP-1 cells (Fig. 6B). In addition, bufalin induced morphological changes in THP-1/TPA cells that were similar to those in bufalin-treated parental cells (data not shown). Bufalin also induced a decrease in the level of c-myb transcripts; relative c-myb mRNA contents in THP-1 and THP-1/TPA cells treated with 30 nM bufalin for 48 hr were 40.2% and 54.1%, respectively. In addition, bufalin caused induction of c-fos and Egr-1 mRNAs in THP-1/TPA cells, although its effect on c-fos expression was reduced at 48 hr after treatment (Fig. 7B). Furthermore, pretreatment of THP-1 cells with the PKC inhibitor H7 revealed no effect on bufalin-mediated accumulation of c-fos mRNA, although the inhibitor prevented TPA-mediated c-fos induction (Fig. 8).

Effect of Bufalin in Low-Na^+ Medium

We have shown that bufalin interacts with the Na^+, K^+-ATPase with similar kinetics to ouabain [3], a specific inhibitor of the enzyme. Most animal cells have a high concentration of K^+ and a low concentration of Na^+ relative to the external medium. The Na^+, K^+-ATPase generates and maintains these ionic gradients. Inhibition of Na^+, K^+-ATPase by bufalin can, thus, result in an increased intracellular Na^+ concentration [14, 15]. To determine whether or not the disruption of the Na^+ gradient was necessary for bufalin-induced changes in proto-oncogene expression, we used a low-Na^+ RPMI 1640 medium, in which the Na^+ was replaced with equimolar choline ions. Although bufalin at concentrations of up to 30 nM apparently showed no effect on the viability of THP-1 cells (Fig. 2A), a dose-dependent cytotoxicity was observed at a concentration of 50 nM or higher in the complete medium (Fig. 3A). For example, incubation of the cells with 100 nM bufalin for 48 hr resulted in a decrease in cell viability to approximately 31% (Fig. 3A). On the other hand, although cultivating THP-1 cells in the low-Na^+ medium for 48 hr caused a decrease in cell viability to approximately 70%, bufalin appeared to have no further effect up to 100 nM (Fig. 3A). In addition, no morphological change was noted in the cells treated with bufalin in the low-Na^+ medium (data not shown).

When unstimulated THP-1 cells were cultivated in the low-Na^+ medium, no apparent change was observed in a steady-state level of c-fos mRNA up to 48 hr. c-myb mRNA, on the other hand, decreased significantly even 1

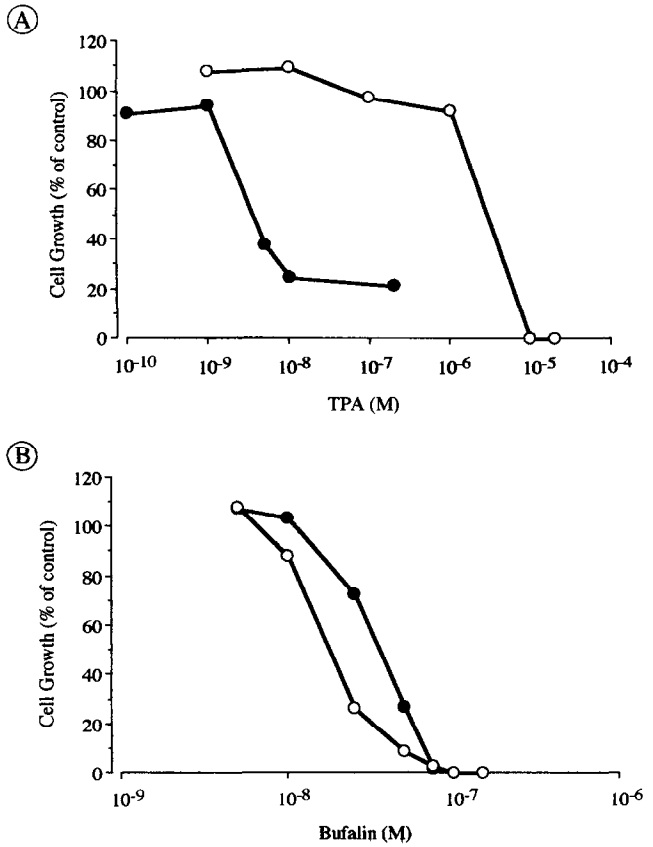

FIG. 6. Effect of TPA and bufalin on growth of THP-1 and THP-1/TPA cells. THP-1/TPA cells were established and cultivated as described in Experimental Procedure. THP-1 (closed circle) and THP-1/TPA (open circle) cells (1×10^5 cells/mL) were incubated in the presence of TPA (A) or bufalin (B) at the concentrations indicated for 3 days. Cell growth was assessed by XTT formazan assay. The mean of 3 determinations is illustrated.

hr after the medium was changed (data not shown). However, a detectable level of c-myb mRNA was still observed in cells cultivated for 24 hr in the low-Na^+ medium (Fig. 3B). Thus, effects of bufalin on proto-oncogene expression in the low-Na^+ medium were determined after 24-hr incubations; in these experiments, bufalin was added just after the medium was replaced. The inducing effect of bufalin on c-fos mRNA seen in the complete medium was abrogated almost completely in the low-Na^+ medium, whereas TPA effectively induced c-fos expression regardless of the low extracellular Na^+ concentration (Fig. 3B). Similarly, bufalin did not affect the steady-state level of c-myb mRNA up to 100 nM in the low-Na^+ medium. Again, TPA was effective in decreasing c-myb expression in the low-Na^+ medium (Fig. 3B).

DISCUSSION

The monocytic THP-1 cells offer a unique system for the study of macrophage differentiation. We have demonstrated, here, that bufalin induces morphological and functional maturation of THP-1 cells. The cells ceased proliferation almost completely with bufalin treatment at doses as low as 30 nM. At the same time, bufalin induced respiratory burst and expression of IL-1β mRNA. Bufalin also induced dramatic alteration in cell morphology and adherence to tissue culture plastic. These characteristics are features of differentiated macrophage-like cells [13]. Proto-oncogenes encoding transcription factors are implicated in signal transductions involving cell growth and differentiation [16]. During the bufalin-mediated THP-1 cell differentiation process, c-fos and Egr-1 transcripts increased, and constitutive expressions of c-myb and c-myc mRNAs decreased continuously. In particular, the induction of c-fos mRNA appeared within 3 hr after the treatment with bufalin that obviously preceded the appearance of phenotypic expressions associated with cell differentiation. Similar changes in c-fos expression during the differentiation of monocytic cell lines have been demonstrated in cases that used other inducers [17–20]. It is interesting to note that transient induction of Egr-1 mRNA, peaking at 48 hr, was observed after bufalin stimulation. Induction of this transcription factor has been reported to be a very early event in macrophage differentiation [10, 21–22]. However, it is suggested that Egr-1 may play a role not only in macrophage development, but also in the maintenance and/or regulation of a certain function(s) of the differentiated cells [21]. Thus, Egr-1 induced in the late time period of bufalin-mediated monocytic differentiation could be involved in the expression of differentiated phenotype, and be induced by cytokines produced in bufalin-treated cells. Consequently, it is possible that those changes in proto-oncogene expression are the common response to differentiation stimuli, rather than bufalin-specific phenomena.

PKC, a TPA receptor-mediated signal transduction, is the most documented cascade involving monocytic differentiation [23–27]. We examined, in this study, whether or not differentiation signals elicited by bufalin overlap with those from TPA. For that purpose, we used THP-1/TPA cells, which are not only unresponsive to the growth-inhibitory effects of TPA, but also attenuate the oncogenic response to this agent. Bufalin effectively inhibited growth of THP-1/TPA cells and altered proto-oncogene expression in a manner similar to that of THP-1 cells. In addition, the nonselective protein kinase inhibitor H7 inhibited TPA-mediated c-fos induction; however, this inhibitor showed virtually no effect on bufalin-mediated c-fos induction. These observations indicate that signal cascades elicited by bufalin can be separable from those of TPA, although the biochemical nature of THP-1/TPA cells remains to be determined.

Ouabain specifically binds the extracellular domain of the α-subunit of Na^+, K^+-ATPase and, thus, inhibits enzyme activity [14]. Na^+, K^+-ATPase catalyzes the active transport of 3 Na^+ out of the cell and 2 K^+ into the cell. We have reported that ouabain also induces functional maturation in K562 cells; however, its effective concentration is

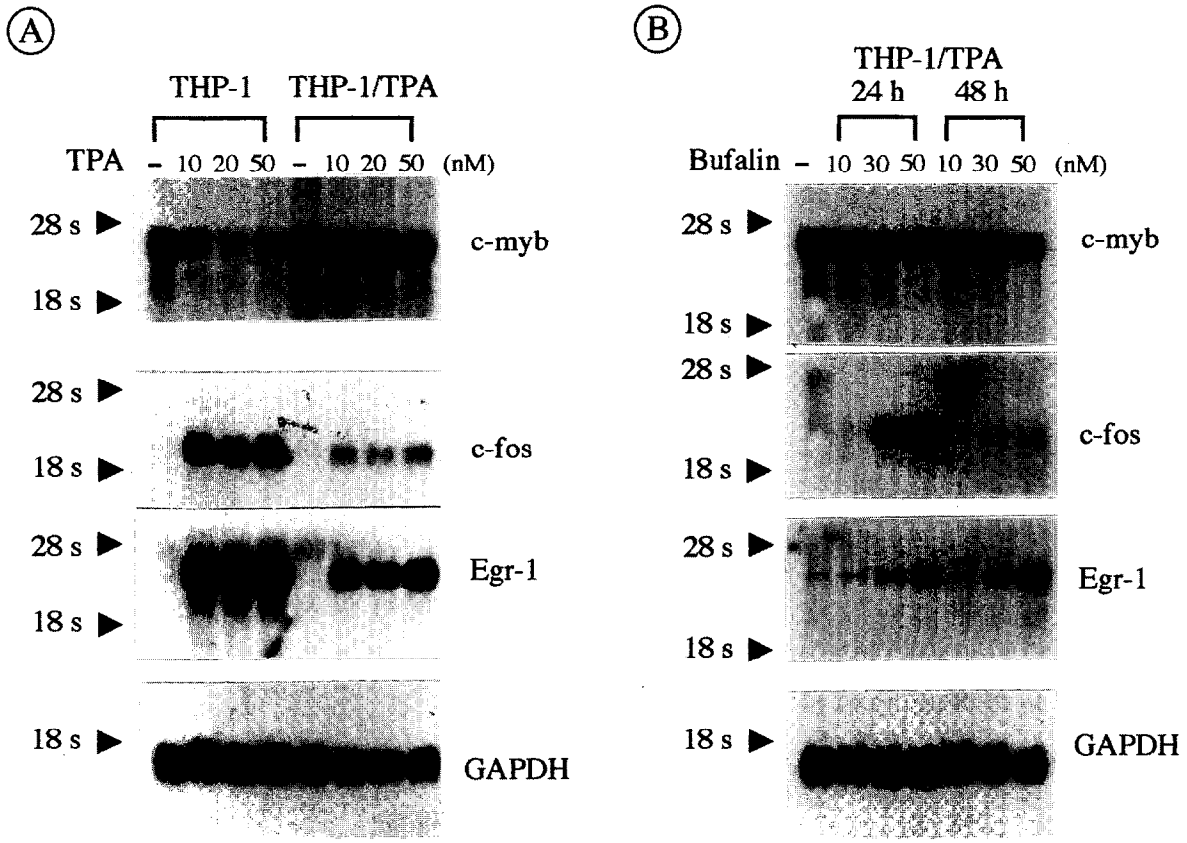

FIG. 7. Effect of TPA and bufalin on proto-oncogene expression in THP-1/TPA cells (A) THP-1 or THP-1/TPA cells were incubated in the presence of TPA at the concentrations indicated for 6 hr. (B) THP-1/TPA cells were incubated in the presence of bufalin at the concentrations indicated for 24 or 48 hr. Proto-oncogene expression was determined as described in the legend to Fig. 4 and Experimental Procedure.

10-fold higher than that of bufalin [3]. The present study revealed that ouabain was as effective as bufalin on the induction of respiratory burst in THP-1 cells. In addition, ouabain produced changes in proto-oncogene expression identical to that seen after bufalin treatment. These results suggest that Na^+, K^+-ATPase is a common target of these cardiotonic steroids and enzyme inhibition is an initial event involving bufalin-mediated leukemia cell differentiation. To delineate this hypothesis, we have tested the effect of bufalin in the low-Na^+ medium, because the medium may suppress passive Na^+ influx, thereby inhibiting the increase in intracellular Na^+ concentration caused by Na^+, K^+-ATPase inhibition. The cytotoxic effect of a high dose of bufalin (100 nM) seen in the complete medium was virtually canceled in the low-Na^+ medium. Furthermore, bufalin failed to induce alteration of proto-oncogene expression in the low-Na^+ medium. Increased intracellular Na^+ concentration may lead to Ca^{2+} influx by the Na^+/Ca^{2+} exchange system [28, 29]. We, therefore, tested $^{45}Ca^{2+}$ influx in bufalin-treated THP-1 cells; however, bufalin did not increase the radioactivity associated with the cells (data not shown). Taken together, these observations suggest that the biological action of bufalin, such as possible changes in proto-oncogene expression, is attributable to passive Na^+ entry into the THP-1 cells.

It is noteworthy that disruption of the cation levels appears to be coupled to leukemia cell differentiation [28, 30–34]. However, the present study, in conjunction with our previous findings [3], reveals direct evidence that this ubiquitous enzyme has a role in cell differentiation, although downstream of the Na^+, K^+-ATPase-mediated differentiation signal remains to be determined. Because a specific cardiotonic steroid-binding site of high affinity and selectivity was shown to be conserved in Na^+, K^+-ATPases in all animal species during evolution [14], the presence of endogenous Na^+, K^+-ATPase inhibitors (endogenous digitalis-like factors or EDLFs) has been postulated [15]. In fact, Hamlyn and colleagues [35] have found that their preparation of an EDLF that was isolated from human plasma is indistinguishable from plant-origin ouabain. Although this observation is controversial because the biosynthetic pathway of ouabain in animals has yet to be determined, several lines of evidence suggest that the ouabain-like compound is of endogenous origin [35–37]. Our present hypothesis and the recent findings that demonstrated bufalin-like factor(s) in human plasma [38] also propose that EDLFs would function as endogenous cell differentiation factors.

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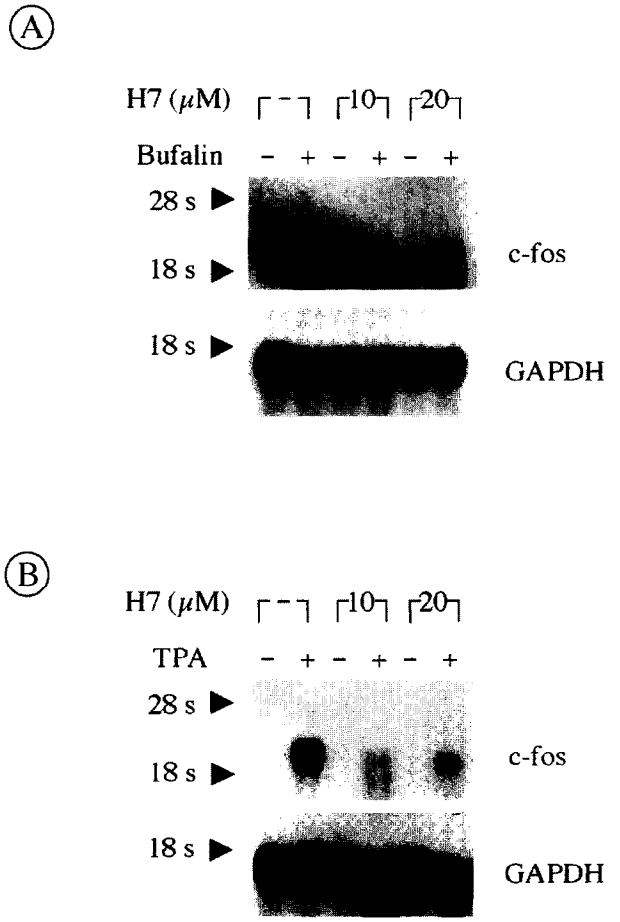

FIG. 8. Effect of H7 on bufalin-mediated induction of c-fos mRNA in THP-1 cells. Cells pretreated with H7 at the concentrations indicated for 1 hr were incubated with 30 nM bufalin (A) and 10 nM TPA (B) for 48 hr and 6 hr, respectively. Expression of c-fos proto-oncogene was analyzed on Northern blots as described in the legend to Fig. 4 and Experimental Procedure.

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