Physiologic Importance of In Vitro Studies with Autacoids

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Autacoids are effector molecules that act on cells close to the locale of their synthesis [1]. In some cases autacoids also have the potential to enter the circulation, so as to affect cells in other tissues [1]. Early studies were concerned with eicosanoids, lipid acids derived from arachidonic acid via either Cyclooxygenase (COX) or Lipoxygenase (LOX) [2]. Prostaglandins (PGs), products of COX, initially isolated from seminal fluid, were subsequently found in numerous tissues. Many different classes of autacoids are presently being studied, including angiotensin, norepinephrine, dopamine, nitric oxide, endothelins, Insulin-Like-Growth Factor (IGF), Epidermal Growth Factor (EGF) and Fibroblast Growth factor (FGF), to name a few. Of particular interest to this report, is the use of cultured animal cells to study the effects of autacoids.

A question that arises in these regards is whether observations of the effects of autacoids in vitro are physiologically relevant. In initial cell culture studies, investigators were concerned whether differentiated cells actually dedifferentiated when cultured, and became fibroblasts [3]. However, the problem of fibroblast outgrowth of primary cultures was subsequently found to be the consequence of the use of serum in the culture medium which gave a select advantage to fibroblasts and in some cases was actually toxic to differentiated cells. When using defined medium lacking serum, differentiated cells may be grown selectively in primary cultures in the absence of fibroblasts. Under these conditions, physiologically relevant effects of autacoids may be observed [4]. Similar responses may be difficult to discern in vivo, especially when the physiologic environment is rapidly changing.

Presently, a number of in vitro cell culture systems are available, which are highly differentiated, and closely resemble normal cells present in their tissue of origin [5]. A number of growth studies have been conducted with these differentiated cell culture systems in hormonally defined media [3,6]. In the absence of serum, the growth requirements of differentiated cells have been found to differ, in a tissue-specific manner. A number of autacoids have been observed to stimulate growth in defined medium, also in a tissue specific manner, suggestive that the cells may have similar requirements in vivo. One example of such an autacoid is, putrescine, a growth factor for B104 rat neuroblastoma cells, as well as primary neurons [7,8]. Consistent with an in vivo requirement, putrescine is detectable in brain [9] and has been reported to stimulate brain neurogenesis, as well as neurite outgrowth [10]. Hormones may similarly stimulate the growth of cultured animal cells, as exemplified in the case of Follicle Stimulating Hormone (FSH), which is a growth factor for rat Sertoli cells in culture [4]. This makes physiologic sense, because after FSH is produced by the gonadotrophic cells in the anterior pituitary [11], FSH acts on Sertoli cells within the seminiferous tubules of the testis, so as to promote spermatogenesis [11]. Increases in the number of Sertoli cells are observed following the production of FSH, in addition to increases in the level of the FSH receptor.

Another class of autacoids, PGs, stimulate the growth of a number of cell lines in vitro [4,12]. For example, the growth of the dog kidney tubule epithelial cell line MDCK is stimulated by either PGE, or PGE2 [13]. Consistent with the physiologic significance of the growth stimulatory effects of PGE, and PGE2, is the observation that the growth of primary baby mouse kidney cells (taken directly from the animal) is stimulated by the same PGs [14]. The growth of epithelial cells derived from a number of other tissues is similarly stimulated by PGE2, including mammary epithelial cells [15], corneal epithelial cells [16], and intestinal epithelial cells [17]. Indeed, increased growth of colon cancer cells has been attributed to increased production of PGE2 by the tumor [18].

PGs have a number of functional affects. In the kidney, PGs affect vascular tone, as well as epithelial transporters [19]. However, the functional effects of PGs observed in vitro, are often difficult to validate in vivo, due to complexity of organs such as the kidney. The kidney is composed of numerous nephrons, each of which has distinct segments. Due to the convoluted structure of the nephrons, they cannot be purified simply by cutting out kidney slices. Instead, individual nephron segments are isolated. Using such individual nephron segments, the effects of PGE2 on water reabsorption in the CD has been proposed to be mediated by Gi-coupled EP3 receptors (localized in this nephron segment). Indeed, the EP3 agonist sulprostone antagonizes the effect of AVP on water reabsorption by the kidney [21]. This is apparently a major effect, as Non steroidal Anti-inflammatory Drugs (NSAIDS) cause water retention [19]. Nevertheless, mice with a targeted disruption of EP3 on water reabsorption by the kidney [21]. This is apparently a major effect, as Non steroidal Anti-inflammatory Drugs (NSAIDS)

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indicated that EP2 and EP4 are involved in Na, K-ATPase regulation [23]. All 4 EP receptors (EP1, EP2, EP3 and EP4) are present both in MDCK cells and primary rabbit RPT cells [24,25]. Evidence for chronic stimulatory effects of PGs, and PGE, on Na, K-ATPase has been obtained in both renal cell types [23,25]. The increased rate of Na,K-ATPase biosynthesis observed in MDCK cells treated with PGE, have been explained by transcriptional regulation of the Na,K-ATPase β subunit gene (atp1b1) via Prostaglandin Response Elements [23,26,27]. Evidence for the involvement of both Gq-coupled EP1 and Gs-coupled EP2 in the transcriptional regulation of atp1b1was obtained initially [23]. Subsequently, similar transcriptional studies were conducted in the primary rabbit RPT cells, and additional evidence was obtained for EP4 involvement [25].

Although PGE, and PGE, are also growth stimulatory factors for these renal cells, our studies with genetic variants of MDCK indicate that distinct CAM mediated mechanisms are involved in mediating the stimulatory effects of these PGs on growth [28]. Studies concerning growth and transport are still lacking in intact animals. Nevertheless, the results with Na, K-ATPase are consistent with previous studies of Na,K-ATPase in isolated proximal convoluted and distal tubules [29].

In the cell culture studies described above, similar results were obtained with primary cultures obtained directly from the animal and an established cell line [25,26]. When experiments are conducted with established cell lines in vitro, in the absence of other modes of confirmation, caution must be taken with regards to the conclusions. Animal cell lines may have changed while being established in culture. In addition, cell function may be affected by immortalizing oncogenes. Indeed, following immortalization with SV40 early region genes, rabbit RPT cell lines had considerably lower rates of sugar uptake than primary RPT cell cultures, and did not grow in glucose free medium (unlike primary cultures), indicating the loss of gluconeogenic capacity [30].

The cell culture environment is another concern when evaluating the results obtained in vitro. Imperfections in the in vitro environment (which do not effectively simulate the in vivo situation) may result in cellular adaptations, which are necessary for cell proliferation and survival. In this case, concern must be raised about the physiologic relevance of effectors and signaling pathways in studies with such immortalized cells.

To summarize, in vitro studies concerning the effects of autacoids on differentiated animal cells have potential to make significant contributions to our understanding their signal transduction pathways in vivo. The use of hormonally defined culture medium makes it possible to study the effects of autacoids at physiologically relevant concentrations, and to maintain cultures in their differentiated state. Conclusions obtained with immortalized cell lines should be assessed using other model systems including primary cultures, as signaling pathways and hormonal responses may be altered as a consequence of cell immortalization. With these constraints in mind, in vitro studies with differentiated cells in defined culture conditions have a great potential to further our understanding of the in vivo situation, and provide an intelligent rationale for therapeutics.

References


