Distribution of mRNA Encoding Three \( \alpha_2 \)-Adrenergic Receptor Subtypes in the Developing Mouse Embryo Suggests a Role for the \( \alpha_{2A} \) Subtype in Apoptosis

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SUMMARY

\( \alpha_2 \)-Adrenergic receptors (\( \alpha_2 \)-ARs) respond to norepinephrine and epinephrine to mediate diverse physiological effects. Using \textit{in situ} hybridization, the expression pattern of the mRNA encoding the three \( \alpha_2 \)-AR subtypes (\( \alpha_{2A} \), \( \alpha_{2B} \), and \( \alpha_{2C} \)) was examined in the mouse embryo. The mRNA encoding the three subtypes was first detected at stage 9.5 days postcoitus (d.p.c.) for the \( \alpha_{2A} \)-AR (coincident with norepinephrine availability), 11.5 d.p.c. for the \( \alpha_{2B} \)-AR, and 14.5 d.p.c. for the \( \alpha_{2C} \)-AR subtype. The mRNA encoding the \( \alpha_{2A} \)-AR subtype shows both the earliest and the most widespread expression pattern, including developing stomach and cecum, many craniofacial regions and areas in the central nervous system. Strikingly, the \( \alpha_{2A} \)-AR mRNA is expressed in the interdigital mesenchyme between stage 12.5 and 14.5 d.p.c. in parallel with digit separation, raising the possibility that the \( \alpha_{2A} \)-AR might contribute to the apoptotic events underlying this process. To test whether \( \alpha_{2A} \)-AR can signal apoptotic events, the \( \alpha_{2A} \)-AR subtype was introduced into two mouse mesenchymal cell lines, C3H/10t and NIH-3T3; expression of the \( \alpha_{2A} \)-AR correlated with accelerated apoptosis, as detected both by the TUNEL assay and the loss of cell viability. In contrast to the wide distribution of mRNA encoding the \( \alpha_{2A} \)-AR subtype, the \( \alpha_{2B} \)-AR mRNA was detected only in the developing liver and was most readily detectable between 11.5 and 14.5 d.p.c., when the liver is the principal site of hematopoiesis. The \( \alpha_{2C} \)-AR mRNA is detected in the nasal cavity and cerebellar primordium only at \( \geq \)14.5 d.p.c. These studies represent the first characterization of the temporal and spatial expressions of the \( \alpha_{2A} \)-AR, \( \alpha_{2B} \)-AR, and \( \alpha_{2C} \)-AR subtypes during embryogenesis and provide important insights concerning the loci and possible roles of \( \alpha_2 \)-AR-mediated regulation of physiological processes during the developmental program.

\( \alpha_2 \)-ARs in adult animals and humans respond to EPI and NE to modulate metabolic effects in adipose, transepithelial Na\(^+\) and water transport in renal and intestinal epithelial cells, suppression of insulin release from \( \beta \) cells of the pancreas, and attenuation of neurotransmitter release in the central and peripheral nervous systems (1). Consequences of activation of \( \alpha_2 \)-AR in the central nervous system include lowering of blood pressure, sedation, enhanced anesthesia, suppression of pain perception, and suppression of epileptogenesis (2).

Recently, \( \alpha_2 \)-ARs have been demonstrated to represent a family of three subtypes based on pharmacological (3) and molecular cloning (4–7) strategies. Northern and \textit{in situ} hybridization analyses have identified mRNA expression of all three \( \alpha_2 \)-AR subtypes in a wide variety of tissues and organs that correspond to loci of known \( \alpha_{2A} \)-AR regulated physiological functions, such as the central nervous system (8, 9), adrenal gland, cardiovascular system, and intestine (3). In contrast, virtually nothing is known about the localization and physiological roles of the AR subtypes in early development.

Recent findings from Thomas et al. (10) and Zhou et al. (11) indicate that the enzymes responsible for NE synthesis are moderately detectable at 8.5 d.p.c. (the earliest time point examined). The catecholamines dopamine and NE are first detectable at days 9.5 and 10.5 d.p.c., respectively, and EPI is consistently detected in fetuses older than 13.5 d.p.c. (10). NE and EPI activate \( \alpha_2 \)-ARs. Because the lack of NE synthesis leads to embryonic lethality (10), the possibility arises that particular \( \alpha_2 \)-AR subtypes may play important roles during development.

As a first step in exploring potential functions for \( \alpha_2 \)-AR in early embryonic development, we identified the distribution of the mRNA encoding \( \alpha_{2A} \)-AR, \( \alpha_{2B} \)-AR, and \( \alpha_{2C} \)-AR subtypes in the early developing mouse embryo through the use of \textit{in situ} hybridization and molecular cloning strategies.
situ hybridization techniques. In addition, when we observed that the distribution of mRNA encoding the α2A-AR subtype was detected temporally and spatially with the programmed cell death of mesenchymal cells during the digit formation, we tested the hypothesis that the α2A-AR is capable of inducing or accelerating apoptosis in mesenchymal cells. The ability of the α2A-AR to accelerate apoptosis in these cells reveals a heretofore unappreciated regulatory consequence of α2A-AR activation in mammalian systems.

Materials and Methods

Embryo collection and fixation. The hybrid offspring between 129/SvEv and C57Black mice were used as mating pairs. Other embryos, derived from mating of 129/SvEv purebreds, B6D2 F1 hybrids, and ICR mice, were also evaluated for comparison of mRNA expression patterns. The pattern of the α2A-AR subtype expression was indistinguishable in these varying genetic backgrounds. Embryos harvested at 8.5 and 9.5 d.p.c. were fixed with 4% paraformaldehyde at 4°C for 2 hr. Embryos harvested at 12.5, 13.5, 14.5, 15.5, and 16.5 d.p.c. were perfused with cold 4% paraformaldehyde, fixed overnight, transferred to 100% methanol, and stored at −20°C.

Whole-mount in situ hybridization. Digoxigenin-labeled cRNA probes encoding antisense (signal) and sense (control) templates were synthesized from templates representing the nearly full-length coding sequence of α2A-AR, α2B-AR, and α2C-AR genes, as described previously (9). Whole-mount in situ hybridization was performed as described previously (12) with minor modifications of hybridization and washing temperatures as outlined briefly below. After overnight hybridization at 70°C, the hybridization temperature was slowly reduced to 55°C and maintained at 55°C for 2 hr. The washing was performed at 55°C before RNase A treatment and 50°C after RNase treatment. After whole-mount hybridization, the embryos were washed with PBS (1× = 2.68 mM KCl, 1.47 mM KH₂PO₄, 136.9 mM NaCl, 8.06 mM Na₂HPO₄·7 H₂O, and 5.6 mM glucose, pH 7.4) and transferred into PBS containing 20% sucrose. Some embryos were sectioned after the hybridization and washed steps.

In some cases, cultured cells expressing α2A-AR, α2B-AR, and α2C-AR subtypes were injected into the brain cavity of early embryos to confirm the ability of the mRNA probes to penetrate and identify the relevant mRNA in a receptor subtype-specific manner. These control studies both validated the selectivity of the probes and ensured that the lack of detectable endogenous mRNA expression for a given α2-AR subtype at certain embryonic stages was not a result of technical limitations in mRNA detection.

Sectioning and evaluation of apoptosis in the embryo. After whole-mount in situ hybridization, the tissue of interest was post-fixed with 4% paraformaldehyde for 1 hr and sectioned with a frozen microtome to the thickness of 60 μm. A hallmark of apoptotic cells is DNA fragmentation, leading to the appearance of high concentrations of 3'-OH ends of single- and double-stranded DNA; these ends are detected by terminal deoxynucleotidyl transferase-catalyzed digoxigenin-dUTP end labeling of these 3'-OH ends (TUNEL assay). Digoxigenin labeling is revealed using a fluorescein-conjugated antidigoxigenin antibody fragment that lacks the Fc portion of the antibody (Apop Tag Plus Kit; Oncor, Gaithersburg, MD). The TUNEL assay was performed according to the instructions of the manufacturer, except for the following modifications: (a) sections were rinsed twice with 0.2% Triton X-100 in PBS for 15 min before the reaction, (b) the reaction was performed in 54 ml of working-strength terminal deoxynucleotidyl transferase enzyme at 37°C overnight, (c) sections were rinsed twice with STOP/WASH buffer at 37°C for 20 min twice and with 2× standard saline citrate for 30 min three times, and (d) the anti-digoxigenin-peroxidase incubation was prolonged to 2–4 hr and washed four times for 30 min each.

Heterologous receptor expression and evaluation of apoptotic signals in cultured cells. To test the hypothesis that α2A-AR are capable of inducing or accelerating apoptosis in mesenchymal cells, we introduced the α2A-AR into two cultured cell lines derived from the mouse embryonic mesenchyme (C57/10Hg and NIH 3T3) through electroporation of a cDNA encoding the epitope-tagged α2A-AR subtype (13). Cells were cultured in Eagle's modified medium plus 10% fetal calf serum. For electroporation, 1.5 × 10⁶ cells were in 0.4 ml of OPTI-MEM (GIBCO BRL, Gaithersburg, MD) were incubated with 10 μg of α2A-AR cDNA and/or 10 μg of pGREEN LANTERN cDNA (GIBCO BRL) and exposed to 280 V/975 μF in a Gene Pulser II (BioRad, Hercules, CA). Cells were then incubated in Eagle's modified medium plus 10% fetal calf serum for various times (see figure legends) in the absence (control) or presence of 10 μM UK 14304, an α2A-AR agonist, or yohimbine, an α2A-AR antagonist. Drug-containing and control media were changed every 12 hr. Control experiments comparing the expression of epitope-tagged α2A-AR, identified immunocytochemically, and GFP, detected by microscopy with 610-nm illumination, confirmed that there is a 100% coincidence of coexpression of these two proteins when cotransfected via the procedures used in this study. Thus, after cotransfection of cDNAs encoding GFP and α2A-AR, detection of expression of GFP can be used as a marker for cells expressing the cDNA encoding the α2A-AR.

Cells were harvested in PBS and fixed in 1% paraformaldehyde for 15 min at room temperature. Cells were then washed by centrifugation at 800 rpm for 5 min and then resuspended in 70% ethanol. The cell sample was stored at −20°C. The expression of epitope-tagged α2A-AR was demonstrated by immunofluorescence microscopy after incubation with the 12CA5 monoclonal antibody directed against the hemagglutinin epitope engineered into the amino terminus of the α2A-AR and a Cy3-conjugated donkey anti-mouse secondary antibody, as described in detail previously (14).

Apoptotic events were measured in control versus α2A-AR-expressing cells using the TUNEL assay as described above. Two alternative strategies for cell handling were used, with comparable findings obtained. Cells were either plated onto coverslips and examined immunocytochemically for α2A-AR expression and then via the TUNEL assay for apoptosis or they were harvested, fixed, and then examined in suspension for α2A-AR expression and apoptosis, after which the cells were applied to microscope slides and sealed under coverslips using Permount.

Photography. Photography for the whole-mount embryos was performed under an Olympus SZH10 dissecting microscope. Photography for the whole-mount embryos was performed under an Olympus SZH10 dissecting microscope. Fig. 1. Schematic of the relative onset of expression of mRNA encoding α2-AR subtypes revealed by in situ hybridization in comparison with the expression of mRNA encoding TH and DJH in the developing mouse embryo. Synthesis of the catecholamine NE occurs via the hydroxylation of tyrosine, via TH, to L-3,4-hydroxyphenylalanine (L-DOPA); aromatic l-amino acid decarboxylase [or DOPA decarboxylase (DCO)] modifies this intermediate to dopamine, which, via dopamine β-hydroxylase, is converted to NE. EPI can be synthesized from NE by the enzyme phenylethanolamine-N-methyltransferase. «Data shown for TH and DJH summarize findings reported in Thomas et al. (10) and Zhou et al. (11).»
raphy for cultured cells and sectioned embryos was performed under a Zeiss Axioplan microscope. All photographs were made using Kodak Ektachrome 160 Tungsten film.

Results

Using whole-mount in situ hybridization strategies with digoxigenin-labeled cRNA probes, the expression pattern of the distinct mRNAs encoding the α2A-AR, α2B-AR, and α2C-AR subtypes was examined in mouse embryonic stages 8.5–16.5 d.p.c. At later stages of development, some of the hybridized embryos also were sectioned using a cryostat to permit more detailed evaluation of the pattern of expression of mRNA encoding the α2A-AR subtypes.

Temporal Expression

Fig. 1 summarizes, via a schematic time-line, the onset of the expression of the mRNA for each α2-AR subtype during embryogenesis. For comparison, the temporal expression of mRNAs encoding TH and ΔβH, enzymes responsible for the synthesis of NE, an endogenous ligand for α2-AR, also are shown (10, 11). The mRNA encoding the α2A-AR subtype was detected earliest and could be identified readily in the 9.5 d.p.c. embryo of the mouse, a time that parallels the detection of catecholamines in the brain cavity of embryonic mice (10). The mRNA encoding the α2B-AR subtype was detected only over a narrow time frame during development (11.5–14.5 d.p.c.). The mRNA encoding the α2C-AR subtype...
was not detected until 14.5 d.p.c. and was still evident at 16.5 d.p.c.

Spatial Expression

$\alpha_{2A}$-AR subtype. The expression of mRNA encoding the $\alpha_{2A}$-AR, first detected at 9.5 d.p.c., was distributed in the developing somites and in scattered cells covering the neural tube. The segmental expression of the $\alpha_{2A}$-AR in the somites lasts until 14.5 d.p.c. but is no longer detected in $\geq15.5$ d.p.c. embryos (Fig. 2). However, after 10.5 d.p.c., the $\alpha_{2A}$-AR mRNA can be detected in many regions, including a variety of craniofacial areas and developing limbs. The regional distribution of the $\alpha_{2A}$-AR through development is given (see Figs. 2–5). Fig. 2 provides a whole-embryo view at days 9.5 (Fig. 2A) and 10.5 (Fig. 2B) d.p.c. and a view of bisected embryos at 14.5 d.p.c. (Fig. 2, D–F). Also shown is the regional expression of mRNAs encoding the $\alpha_{2A}$-AR subtype at 11.5–14.5 d.p.c. (see Figs. 3–5).

In craniofacial areas of 10.5–13.5 d.p.c. embryos, $\alpha_{2A}$-AR mRNA is prominently expressed in the maxillary arch, hyoid arch (Figs. 2B and 3A), mesenchyme cells covering the telencephalon, brain mesoderm (Figs. 2A and 4A), and mesenchyme condensation forming future falx cerebri (Figs. 3C and 4B). In embryos harvested from 10.5–14.5 d.p.c. embryos, $\alpha_{2A}$-AR mRNA is detected in mesenchyme of the nasal septum next to the posterior naris (Fig. 3, B, C, and F). From 13.5 to 16.5 d.p.c., this expression of $\alpha_{2A}$-AR mRNA in the mesenchymal cells spreads to all areas of the nasal cavity, which parallels, temporally, the folding and expansion of this cavity. At the lower and central sides of the anterior naris, expression of $\alpha_{2A}$-AR mRNA is first detected at 11.5 d.p.c. (Fig. 3, C and F) and then becomes very prominent in embryos harvested at 12.5 and 13.5 d.p.c. (Fig. 3, B–E).

The mRNA encoding the $\alpha_{2A}$-AR subtype also is detected in other craniofacial areas, such as the external auditory meatus and the condensation of perioptic mesenchyme (ectomeninx), which subsequently differentiates to form the sclera in 11.5 and 12.5 d.p.c. embryos (Fig. 3E). Expression of $\alpha_{2A}$-AR

Fig. 3. Endogenous $\alpha_{2A}$-AR mRNA distribution in craniofacial areas of the mouse embryo as detected through in situ hybridization. A, 10.5 d.p.c. embryo showing $\alpha_{2A}$-AR mRNA distribution in the maxillary arch (Mx) and hyoid arch (HA) [mandibula (Md)]. F, forelimb. B, 12.5 d.p.c., medial view of the head region after midline bisection of the embryo; shown is the left half. Arrowhead, expression of $\alpha_{2A}$-AR mRNA in the nasal septum. C, 13.5 d.p.c., front view of the head showing expression of $\alpha_{2A}$-AR mRNA in tissue surrounding nasal septum (S) [falx cerebri (FC)]. D, 14.5 d.p.c., medial view after midline bisection of the embryo; shown is the left side. Arrowhead, expression of $\alpha_{2A}$-AR mRNA in the nasal septum (S). E, 14.5 d.p.c., lateral view showing expression of $\alpha_{2A}$-AR mRNA in the external auditory meatus (EAM) and condensation of perioptic mesenchyme (ectomeninx). F, 13.5 d.p.c., bottom view of the head after lower jaw and tongue were removed, showing expression of $\alpha_{2A}$-AR mRNA in tissue around the nasal septum at position of the posterior naris (P).
mRNA also is noted in the tissues covering the olfactory bulb from 10.5 and 16.5 d.p.c. (Fig. 3) and in the submandibular gland in embryos between 14.5 to 16.5 d.p.c. (data not shown).

In addition to its craniofacial distribution, mRNA encoding the α₂A-AR subtype is detected in mesoderm of the back of the cervical region in embryos harvested between 10.5 and 12.5 d.p.c. (Fig. 4A). The expression of α₂A-AR mRNA also is noted between 10.5 and 14.5 d.p.c. in the developing cecum (a limited area of midgut loop within the physiological umbilical hernia; Figs. 4C and 5), in the stomach between 11.5 and 12.5 d.p.c. (Fig. 4C), and in the genital tubercle in embryos harvested at 14.5 and 15.5 d.p.c. (data not shown).

A striking and unexpected finding of this study was the detection of α₂A-AR mRNA in the mesenchyma of the interdigital areas in the developing limbs between 12.5 and 14.5 d.p.c. of embryonic development (Fig. 6), closely paralleling the apoptotic regression leading to digit separation. This interdigital expression of α₂A-AR mRNA is significantly intensified at the developmental stage of 13.5 d.p.c. (Fig. 6B) in comparison to 12.5 d.p.c. (Fig. 6A). At 14.5 d.p.c., α₂A-AR mRNA is mainly detected at the base and remaining web of the nearly separated digits, as well as at newly forming finger joints (Fig. 6C). The α₂A-AR mRNA is no longer detected in the 15.5 d.p.c. limb, a time when digit separation is fully completed (data not shown). We also observed that α₂A-AR expression in the forelimb (Fig. 6, A and C) is always slightly more advanced than that of the hindlimb, as can be
seen in a comparison of $\alpha_2$-AR mRNA expression at stages 12.5 d.p.c. (Fig. 6A) and 14.5 d.p.c. (Fig. 6C). This temporal pattern is, again, consistent with the time course of limb morphogenesis (15–17). Sectioning of the limb after in situ hybridization reveals that only interdigital mesenchyme expresses $\alpha_2$-AR mRNA (Fig. 6, D and E). After sectioning, a hollowed indentation is detected between mesenchymal cells that have expanded as the embryo gets older, presumably as a result of mesenchyme regression after apoptosis (Fig. 6, D and E). Evaluation of the apoptotic zone in limb sections via the TUNEL assay demonstrates a close spatial correlation between cells undergoing apoptosis and cells expressing $\alpha_2$-AR mRNA (Fig. 7).

The correlation between expression of $\alpha_2$-AR mRNA and mesenchymal regression in digit separation suggested the possibility that the $\alpha_2$-AR induces apoptosis in embryonic mesenchymal cells. To evaluate whether $\alpha_2$-ARs are capable of evoking apoptotic events, two mouse embryonic mesenchymal cell lines, C3H/10T1/2 and NIH-3T3 cells, were used as model systems to determine whether activation of $\alpha_2$-AR can induce apoptosis. A cDNA encoding an epitope-tagged $\alpha_2$-AR (13) was cotransfected via electroporation into cells with a cDNA encoding GFP Lantern. Control studies confirmed that immunodetection of heterologously expressed $\alpha_2$-AR always coincided with GFP expression, so monitoring of GFP expression permitted detection of $\alpha_2$-AR-expressing cells after electroporation. We observed (Fig. 8) that cells expressing $\alpha_2$-AR disappeared more rapidly in response to the $\alpha_2$-agonist UK 14304 compared with control cells (non-GFP-expressing cells) cultured in the same dish, implying that the $\alpha_2$-AR-expressing cells were excluded from the cell population, likely via apoptosis, due to activation of the transfected $\alpha_2$-AR receptor. To test the correlation between apoptotic events and $\alpha_2$-AR expression more directly, C3H/10T1/2 (Fig. 9) or NIH-3T3 (not shown) cells transiently expressing $\alpha_2$-AR cells were incubated in the presence of an $\alpha_2$-agonist, UK14304 (10 $\mu$M) or, alternatively, an $\alpha_2$-AR antagonist, yohimbine, and $\alpha_2$-AR expression, as detected immunochemically, was correlated with apoptotic events detected in the TUNEL assay (see Materials and Methods). As shown in Fig. 9, apoptotic nuclei were detected in $\alpha_2$-AR-expressing cells, identified via the 12CA5 antibody directed against the amino-terminal hemaglutinin epitope.
tag engineered into the α₂A-AR (13). When findings from 500-1000 cells examined in random fields by two investigators blinded to the cellular treatment were tabulated at each time point, it was observed that α₂A-AR-expressing cells manifest an increased appearance of apoptotic nuclei and fragmented cells compared with nonexpressing cells in the same culture dish (Fig. 9C) or control cells never transfected with the α₂-AR cDNA (not shown). Furthermore, treatment with the α₂A-AR agonist seemed to accelerate the apoptotic process. The fact that antagonist treatment delayed but did not entirely prevent the α₂A-AR-dependent increase in apoptotic events is likely due to the known agonist-independent activation of the α₂A-AR, presumably due to agonist-independent conformational changes toward the active state of the receptor (R*) known to occur in a variety of cell types for this receptor subtype (see Ref. 18 and text for discussion).

Fig. 8. Mouse embryonic C3H/10t1/2 cells were cotransfected with cDNA encoding the α₂A-AR and GFP (A–C) or the pCMV4 vector backbone (vector, control) and GFP (D–F), as described in Materials and Methods. At various time points after transfection, the relative density of GFP-expressing cells was examined after culture in minimal essential medium plus 10% fetal calf serum (no drugs added; A and D), medium plus α₂A-AR agonist (10 μM UK 14301; B and E), or α₂-AR antagonist (10 μM yohimbine; C and F). Data demonstrate that the α₂A-AR agonist UK 14304 causes an accelerated loss of GFP-expressing cells and, hence, α₂A-AR-expressing cells; this accelerated decline in α₂A-AR-expressing cells can be delayed, but not eliminated, by culture in the presence of the α₂A-AR antagonist yohimbine. These findings are consistent with the known agonist-independent activation of the α₂A-AR, presumably due to agonist-independent conformational changes toward the active state of the receptor (R*) known to occur in a variety of cell types for this receptor subtype (see Ref. 18 and text for discussion).

Discussion

The current results have revealed that the mRNAs encoding three α₂A-AR subtypes display distinctive temporal and spatial distribution patterns during embryonic development of the mouse. The temporal expression of the α₂A-AR mRNA
coincides with the first detection of the catecholamines, NE and EPI, in the mouse central nervous system (10) and occurs 1 day later than expression of mRNA encoding two critical enzymes in catecholamine biosynthesis, TH and DβH (10, 11). The observation that regional distribution of α2A-AR mRNA occurs in a variety of embryonic areas, including stomach and developing cecum (Figs. 4C and 5C), interdigital mesenchyme (Fig. 6) of developing limbs during the interim of digit separation, many craniofacial areas (Figs. 3–5), and limited regions in the brainstem (Fig. 5D) implies that effects of NE during development in these areas might be mediated by the α2A-AR subtype. Interestingly, expression of α2A-AR corresponds to a number of regions that undergo rapid cell proliferation, including stomach at 11.5 and 12.5 d.p.c. and cecum between 10.5 and 14.5. Because the α2A-AR has been demonstrated to be capable of activating the mitogen-activated protein kinase pathway (19–22), it is possible the α2A-AR modulates critical proliferation events during development.

Because it has been postulated that tissues with high proliferative activity may be more likely to undergo programmed cell death or apoptosis (23), it was of particular interest to note that α2A-AR expression also occurs in some cells that undergo programmed cell death (Fig. 6, A and C), such as the mesenchyme in the interdigital region of the limb bud from 12.5–14.5 d.p.c. (15–17, 23). Our results show that the onset and disappearance of α2A-AR mRNA expression are slightly advanced in the forelimb compared with hindlimb buds (Fig. 6), a temporal expression pattern that matches the time frame of forelimb and hindlimb development (24). This temporal and spatial pattern of α2A-AR mRNA expression in the interdigital areas suggested that α2A-AR may be related to pattern formation of the limb (i.e., apoptotic events responsible for digit separation), perhaps in response to available norepinephrine. Our findings in C3H/10t1⁄2 and NIH-3T3 cells demonstrate that α2A-AR can accelerate apoptotic events, as demonstrated by accelerated cell lysis and appearance of apoptotic nuclei (Figs. 9 and 10). These findings are the first demonstration that mRNA encoding α2A-AR is expressed in apoptotic mesenchyme cells and that α2A-AR can evoke apoptotic signals when examined in a mesenchymal cell preparation after heterologous expression.

Recently, Zou and Niswander (25) demonstrated that introduction of a dominant negative form of BMP receptor, dnBMPR-1B, into developing chick limb resulted in a reduction in apoptosis, truncation of digits, and conversion of scales to feathers, suggesting that the BMP signaling pathway is involved in apoptosis of digit separation. Interestingly, BMP has been shown to be a critical factor in the development of sympathetic (catecholamine-synthesizing) neurons and neuronal regeneration (26, 27). Our findings that the α2A-AR facilitates apoptosis in embryonic mesenchymal cells and that the expression of mRNA encoding the α2A-AR directly parallels interdigital mesenchymal regression suggest that the sympathetic nervous system, regulated in its development by BMP, may play a role in programmed cell death...
essential for digit formation. Even if \( \alpha_{2A} \)-ARs do modulate the apoptotic events that contribute to digit formation, however, these receptor-mediated events cannot be solely responsible for digit formation because altered digits are not noted in mice expressing a mutant \( \alpha_{2A} \)-AR (D79N) that behaves as a functional knockout for this receptor subtype (28). However, we do know that the spatial and temporal expression of mRNA encoding all three \( \alpha_{2} \)-AR subtypes is indistinguishable in wild-type and D79N \( \alpha_{2} \)-AR mice. Thus, functional redundancy mediated by the \( \alpha_{2B} \)-AR or \( \alpha_{2C} \)-AR subtype in digit formation is unlikely; it is more likely that the \( \alpha_{2A} \)-AR, although modulatory, is not essential for this mesenchymal apoptotic event. The molecular signals modulated by \( \alpha_{2A} \)-AR during this window of embryonic development have yet to be clarified.

The temporal and spatial expression of the mRNAs encoding the \( \alpha_{2B} \)-AR and \( \alpha_{2C} \)-AR also is of interest. For example, from the period of 11.5–14.5 d.p.c., the \( \alpha_{2B} \)-AR mRNA is detected in the liver, which serves as a blood-forming organ in embryonic development. This profile of \( \alpha_{2B} \)-AR mRNA expression suggests that the \( \alpha_{2B} \)-AR subtype may play a role in embryonic hematopoiesis.

The current study represents the first comprehensive description of the pattern of expression of \( \alpha_{2A} \)-AR, \( \alpha_{2B} \)-AR, and \( \alpha_{2C} \)-AR subtypes in developing mouse embryo. The subtype-specific temporal and spatial patterns of expression suggest that the existence of \( \alpha_{2} \)-AR subtypes, all of which couple to ways of receptor coupling to adenylate cyclase attenuation and activation. Here we propose that the existence of \( \alpha_{2} \)-AR subtypes, all of which couple to ways of receptor coupling to adenylate cyclase attenuation and activation. Here we propose that the correlation of \( \alpha_{2A} \)-AR mRNA distribution with mesoderm regression, the observation that \( \alpha_{2A} \)-AR can facilitate apoptosis in cultured mesenchymal cells, and the ability of particular G protein-coupled receptors to induce apoptosis in some settings (29, 30) suggest that \( \alpha_{2A} \)-AR may control apoptotic events, such as those leading to digit separation, worthy of future exploration in other cellular settings. The patterns of expression noted for all three subtypes may reveal as-yet-unappreciated roles for all of these \( \alpha_{2} \)-AR subtypes during embryonic development.

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