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Short Communication

Anomalous regulation of β-adrenoceptor signaling in brain regions of the newborn rat

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ABSTRACT

Desensitization, an essential homeostatic response to excessive or continued β-adrenoceptor (βAR) stimulation, is deficient in immature cells. To determine the mechanisms underlying anomalous βAR responses in newborn rats, we administered terbutaline, a β_2AR agonist, on postnatal day 2 and evaluated signaling through adenylyl cyclase (AC) in cell membrane preparations 4 h later. Although a small decrement in isoproterenol-stimulated AC was obtained in the forebrain, robust *sensitization* was seen in the brainstem and cerebellum, in association with heterologous increases in AC catalytic activity: increased basal, dopamine-stimulated and forskolin-stimulated AC. Superimposed on this global increase, there was a small degree of βAR and dopamine receptor desensitization, characterized by decreases in the isoproterenol/forskolin and dopamine/forskolin AC activity ratios. Our results indicate that, in some immature brain regions, βAR desensitization is masked by more substantial increases in the activity of signaling elements downstream from the receptors, resulting in sustained responses in the face of continued receptor stimulation. These effects are likely responsible for the maintenance of βAR activity associated with neurotrophic input during synaptogenesis but may also contribute to adverse effects of βAR agonists used in preterm labor.

In the face of continued or excessive stimulation, cellular homeostasis necessitates desensitization, the suppression of cellular responsiveness. Although agonists acting on β-adrenoceptors (βARs) initially stimulate adenylyl cyclase (AC) and produce corresponding elevations of cyclic AMP, the response plateaus or returns to basal values even if agonist administration is continued, a loss of responsiveness that reflects uncoupling of βARs from their response elements (desensitization) as well as reductions in the concentration of receptors at the cell membrane (downregulation) (Mayor et al., 1998; Palczewski and Benovic, 1991; Tsao and von Zastrow, 2000). Desensitization itself occurs in two distinct ways: homologous desensitization, where effects are restricted to βAR signaling, and heterologous desensitization, where the loss of response involves other receptors and/or components downstream from the receptors, thus compromising any signal mediated through AC (Clark et al., 1989; Goldman et al., 1997; Iwami et al., 1995; Premont et al., 1992; Yamashita et al., 1989).

It is thus surprising that the ability to desensitize βAR responses is not an autochthonous property but rather is acquired during cellular development (review Slotkin et al., 2003). Indeed, immature cardiac, hepatic or neuronal cells exposed chronically to βAR agonists actually show heterologous *sensitization* instead of desensitization, primarily through

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increased expression and augmented function of the G-proteins that couple receptors to AC, as well as induction of AC itself (Slotkin et al., 2003). Because these unusual adaptations are downstream from the receptor, the question remains as to whether desensitization at the receptor level is actually deficient in immature cells or whether receptor desensitization is occurring but is masked by the postreceptor stimulatory processes. Accordingly, in the current study, we examined the short-term regulation of AC-mediated responses after a single exposure of newborn rats to terbutaline, a β_2AR agonist that crosses the blood–brain barrier; by evaluating effects only 4 h after terbutaline administration, we hoped to uncover regulatory events that precede the long-term induction of postreceptor signaling proteins. To evaluate homologous and heterologous mechanisms, we assessed basal AC activity, stimulatory responses to βARs and dopamine receptors and the response to forskolin, which stimulates AC without receptor participation. Finally, because repeated neonatal terbutaline administration shows disparate regional effects on AC regulation, with desensitization in the forebrain but compensatory heterologous adjustments that maintain signaling in the brainstem or cerebellum (Slotkin et al., 2001), we contrasted effects on these same three regions after a single dose of terbutaline.

All experiments were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health and were evaluated and approved by an institutional animal research committee. Six timed pregnant Sprague–Dawley rats (Zivic Laboratories, Pittsburgh, PA) were housed in breeding cages, with a 12-h light–dark cycle and free access to food and water. Pups from all litters were randomized on the day after birth and redistributed to the dams with litter sizes of 10 pups to ensure standardized nutrition and maternal care. On postnatal day 2, one male pup in each litter was given terbutaline hemisulfate (10 mg/kg s.c.; Sigma Chemical Co., St. Louis, MO) and another was given an equivalent volume of saline (1 ml/kg); each litter thus contributed only one control and one terbutaline-treated pup so as to obviate differences in maternal caretaking as a potential confound for treatment effects. Each treatment group thereby contained a total of 6 animals, with each litter contributing only one animal to each group. This dose of terbutaline elicits robust βAR stimulation in the neonatal rat, simulating the effects seen with its use in preterm labor, including cardiac activation and enhancement of lung surfactant synthesis, while maintaining preferential stimulation of the β_2AR subtype (Auman et al., 2002; Garofolo et al., 2003; Kudlacz et al., 1989a,b; Navarro et al., 1991; Slotkin et al., 2001, 2003). For tocolytic therapy in humans, doses typically lie in the range of 0.5 mg/kg/day but can also be as high as 1–2 mg/kg/day (Goldenberg, 2002; Lam et al., 1998). In light of the fact that terbutaline has a much shorter half-life in the rat (Tegner et al., 1984), we used a proportionally higher dose that likely lies at the upper end of potential human exposures but is also of shorter duration than that often used in terbutaline maintenance therapy (Lam et al., 1998, 2001, 2003).

Four hours after treatment, animals were decapitated and the brains were dissected into three regions: the cerebellum (including flocculi) was removed and the forebrain was separated from the brainstem by a cut rostral to the thalamus. All tissues were then frozen in liquid nitrogen and maintained at –45 °C. AC assays were conducted essentially as described in previous papers (Aldridge et al., 2005; Auman et al., 2002; Meyer et al., 2005; Slotkin et al., 2001), so only a brief description will be provided here. Tissues were thawed and homogenized with a Polytron, and cell membranes were prepared and washed by sequential sedimentation at 40,000×*g*. The membrane pellets were dispersed with a smooth-glass homogenizer, and AC activity was determined by enzymatic generation of cyclic AMP which was then measured with radioimmunoassay kits (GE Healthcare, Piscataway, NJ). In addition to measuring basal AC activity, we assessed the response to βAR stimulation (100 μM isoproterenol; Sigma), dopamine receptor stimulation (100 μM dopamine; Sigma) and the response to the direct AC stimulant forskolin (100 μM; Sigma). These concentrations of each stimulant produce maximal responses (Aldridge et al., 2005; Auman et al., 2002; Meyer et al., 2005; Slotkin et al., 2001).

Data are presented as means and standard errors, with treatment effects established by ANOVA (data log-transformed because of heterogeneous variance) encompassing all three variables, treatment, region and AC measure. Lower-order ANOVAs were then conducted in keeping with the interactions of treatment with the other variables followed by Fisher's Protected Least Significant Difference to evaluate individual values that differed between the terbutaline and control groups. Significance for all tests was assumed at $P < 0.05$. For convenience, some data are presented as the percentage change from the corresponding control but all statistical tests were conducted only on the original data. Control values are in Table 1.

In brain regions of control neonates, each stimulant by itself evoked significant increases over basal activity, but there were distinct regional differences in the response (Table 1). Both of the receptor agonists, isoproterenol and dopamine, produced a larger proportional AC increase in the forebrain as compared to the other regions; the response to dopamine was not evaluated in the cerebellum, a region sparse in dopaminergic projections. Similarly, with the direct AC stimulant,

Table 1 – Adenylyl cyclase activity in control brain regions

Measure [a]	Forebrain	Brainstem	Cerebellum
Basal	535 ± 28	1004 ± 31	141 ± 29
Isoproterenol	616 ± 26	1046 ± 48	153 ± 33
Dopamine	694 ± 39	1171 ± 48	—
Forskolin	2574 ± 104	2837 ± 119	217 ± 46

Across all regions, each stimulant caused significant ($P < 0.001$) increases over basal activity (isoproterenol, $F_{1,15} = 20$; dopamine, $F_{1,10} = 520$; forskolin, $F_{1,15} = 4300$). In addition, there were regional differences in the responses. For isoproterenol, the proportional increase was greater in the forebrain than in the other two regions ($P < 0.05$; $F_{2,15} = 4.2$), and, similarly, the increase caused by dopamine was greater in the forebrain than in the brainstem ($P < 0.0003$; $F_{1,10} = 31$); the response to dopamine was not evaluated in the cerebellum. For forskolin, the proportional response followed the hierarchy forebrain > brainstem > cerebellum ($P < 0.0001$; $F_{2,15} = 465$).
[a] pmol/min/mg protein.