Possible Genomic Consequence of Nongenomic Action of Glucocorticoids in Neural Cells

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The nongenomic, rapid effects of glucocorticoid activate multiple intracellular transduction pathways. This review proposes a possible genomic consequence of the nongenomic action of steroids. The genomic actions of hormonal steroids may be twofold: classic genomic and nongenomically induced genomic.

In the 1960s, the intracellular receptors for the steroid hormones were identified and the action of the steroid hormone was attributed to its binding with the receptor, which resulted in the activation of the genome in the cell nucleus as well as changes in the synthesis of new proteins. The genomic theory of steroid hormone action, which has been widely accepted for more than 30 years and is described in most discriminating textbooks as the sole mechanism of steroid hormone action, can satisfactorily account for most metabolic and developmental processes with slow onset and an extended time course. However, the theory met difficulties in explaining the rapid actions of steroid hormones in the body. As a complement to the classic genomic theory, a nongenomic mechanism has been proposed for the rapid action of hormonal steroids and has been widely documented (6, 13, 15, 18). In the present review, the pleiotropic pathways for the intracellular transduction pathways of the rapid action of glucocorticoid (GC) and the possible genomic sequel to the nongenomic action of GC are discussed. If the notion of a genomic sequel to the nongenomic action can be validated by substantial data, the genomic actions of hormonal steroids may be at least twofold: a “classic” genomic action and a nongenomically induced genomic sequel.

Nongenomic action mediated via a putative membrane receptor as a plausible explanation for the rapid actions of the GC

Early observations indicated the existence of nongenomic actions of GCs. It has been observed that a rapid feedback action of GC on the pituitary occurs within 5 min after an increase of the plasma level of GC, that the rapid changes in firing activity of central neurons occurs after iontophoretic application of steroids, and that GC could rapidly inhibit the ascorbic acid uptake in the nerve terminals of the sectioned pituitary. These observations are hardly compatible with the traditional genomic theory of steroid action (5).

We revisited the issue and first proposed a hypothesis in 1987 on the basis of the facts that 1) GC could rapidly modulate neuronal excitability by hyperpolarizing the neuronal membrane and 2) it acted on the neuron from the external surface of the plasma membrane (3, 9) (Fig. 1). This notion envisages a new mechanism for the rapid action of GC on neurons that is mediated by the putative membrane receptor for the steroid hormone. Because the rapid action does not involve the genome, the term “nongenomic mechanism” was introduced. However, the rapid action of GC is not confined to its effect on neuronal excitability; it can also rapidly modulate the neuronal release of arginine vasopressin in hypothalamic slices (11) and secretion of catecholamines in adrenal chromaffin cells as well as in PC12 cell lines. Moreover, it rapidly modulates the uptake of glutamate and glycine in neurons and glia (19). Further biochemical and morphological data substantiate the notion of a membrane receptor of GC on the neuronal plasma membrane, although the entity and identity of the putative membrane receptor for GC remains lacking in spite of the great efforts made in the last decade, including those from this laboratory (2, 5, 8). Indeed, the situation is the same for the putative membrane receptors of all other kinds of hormonal steroids.

Multiple signal transduction pathways are involved in the rapid, nongenomic effects of GC

Because the rapid, nongenomic effects of steroid hormones are well documented, their signal transduction pathways were explored. Recent experimental results indicate that they are remarkably pleiotropic.

\textit{G} protein involvement. Many observations indicated the possible involvement of G proteins in the rapid action of GC. For example, in normal cultured rat pituitary cells, GCs at a physiological concentration rapidly inhibit cAMP production and prolactin release induced by vasoactive intestinal peptide acting through specific GC receptors; GCs act rapidly in vitro to attenuate second-messenger responses to ACTH secretagogues in the rat. However, the precise mechanism underlying the process remains unknown (5).

Binding studies also supported the involvement of G proteins in GC’s rapid actions. It has been found that, in Taricha, \[^{[3]}^{}\mathrm{H}]\text{corticosterone ([^{[3]}^{}\mathrm{H}]B) binding in neuronal membranes is}

enhanced in a concentration-dependent manner by adding Mg\textsuperscript{2+} to the assay buffer. The results are consistent with comparable studies for known G protein-coupled receptors and provide evidence that the putative membrane receptor of B is coupled to G protein (14, 15).
Protein kinase A pathway. It has been shown that cortisol rapidly reduces prolactin release and cAMP and 45Ca2+ accumulation in cichlid fish pituitary in vitro; that dexamethasone (100 µM) could potently suppress the forskolin-stimulated cAMP efflux, ACTH secretion, and proopiomelanocortin expression; and that a pertussis toxin-sensitive GTP-binding protein might, at least partly, participate in the effect (1). It was once suggested (5) that the protein kinase A (PKA) involvement is related to pituitary cells, but recent evidence seems to indicate that, in some cell lines, PKA is also involved in the rapid action of GC.

**FIGURE 1.** Membrane receptor-mediated effects of glucocorticoid (GC) on celiac ganglion neurons. A: BSA-conjugated cortisol (F-BSA) hyperpolarized a celiac ganglion cell in a dose-dependent manner. B and C: the action of F-BSA can be partially blocked by RU-38486. D: cortisol (F) apparently increased the membrane resistance during its hyperpolarizing effect, but when the membrane potential was manually clamped, it decreased the input resistance. The top and bottom tracings show membrane current and potential, respectively. E: in a spontaneously discharging neuron, F-BSA inhibited the discharge of the neuron while hyperpolarizing it. With the pen recorder, only excitatory postsynaptic potentials (upward deflections) and afterhyperpolarizations of the action potentials (downward deflections) were recorded, while the spikes of the action potential were too rapid to be followed. Figure reproduced from Ref. 4 with permission.

**FIGURE 2.** Involvement of protein kinase C (PKC) in GC’s inhibition of nicotine (Nic)-induced Ca2+ influx in PC12 cells. A: cells were preincubated with corticosterone (B) at various concentrations at 25°C for 5 min and then were stimulated with Nic (100 µM). Intracellular Ca2+ concentration ([Ca2+]i) was determined by fura 2 fluorescence. Data are means ± SE (n = 20 cells/data point) of maximal [Ca2+]i over basal or resting values. The IC50 value for B to block the Nic-induced increase in [Ca2+]i was 0.61 ± 0.19 µM. B: effects of B, B-BSA, phorbol 12-myristate 13-acetate (PMA; a PKC activator), Gö-6976 and chelerythrine (PKC inhibitors), and verapamil and ω-conotoxin (Ca2+ channel blockers) on the Nic-induced increase in [Ca2+]i. **P < 0.01 vs. the Nic group. C: effect of PKC inhibitor chelerythrine on PKC activity stimulated by B and PMA. Cells were exposed to different drugs (B, 1 nM; PMA, 100 nM) at 37°C for 5 min after preincubation with or without chelerythrine (100 µM) for 15 min. Then PKC activity in the membrane fractions was assayed with SigmaTECT PKC assay system. Data are means ± SE of 3–5 experiments. **P < 0.01 vs. B group. D: time course of activation of PKC by B. E: dose-dependent activation of PKC by B and B-BSA. ** and ##, P < 0.01 vs. basal. Figure modified from Ref. 16 with permission.
Protein kinase C pathway. It has become clear that the G protein-PKA pathway may not be the sole route for signal transduction of GC's rapid action. The G protein-protein kinase C (PKC) pathway is also involved. Electrophysiological studies demonstrated that cortisol inhibited Ca2+ current in guinea pig hippocampal CA1 neurons via a G protein-coupled activation of protein kinase. In pertussis toxin-treated animals, cortisol inhibition was significantly diminished. Intracellular dialysis with GDPβS or with the pseudosubstrate of PKC (PKC19-31) and PKC inhibitor bisindolylmaleide significantly diminished the cortisol inhibition of the Ca2+ current (7). The specific inhibitor of cAMP-dependent PKA, Rp-cAMPs, had no effect.

We have analyzed the inhibition by GC of intracellular Ca2+ concentration increment (∆[Ca2+]i) induced by various secretagogues in adrenal chromaffin cells as well as in PC12 cells. In the experiments, cells were loaded with fura 2-AM and the intracellular Ca2+ concentration ([Ca2+]i) in single cells was determined by the imaging system. When cultured PC12 cells were treated with acetylcholine, nicotine, bradykinin (BK), or high K+, an immediate and prompt increase in [Ca2+]i (∆[Ca2+]i) was observed. B as well as B-BSA can dose-dependently inhibit the ∆[Ca2+]i (5, 12, 16).

Nicotinic acetylcholine receptors are expressed in PC12 cells, and the inhibition by B of ∆[Ca2+]i induced by nicotine and high K+ can be blocked by verapamil and ω-conotoxin. Nicotine would be expected to raise [Ca2+]i due to Ca2+ entry predominantly through voltage-dependent channels and additionally through nicotinic receptor-associated channels. B also significantly inhibited ∆[Ca2+]i induced by BK. The BK receptors can be categorized into two subtypes: BK1 and BK2. The BK receptor subtype in PC12 cells is exclusively BK2, which is coupled to the Gq/11 family. In addition to intracellular Ca2+ release, BK can also induce extracellular Ca2+ influx through receptor-activated, non-voltage-gated Ca2+ channels. To dissociate the intracellular Ca2+ release and extracellular Ca2+ influx

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**FIGURE 3.** B-induced rapid phosphorylation of mitogen-activated protein kinases (MAPKs) in PC12 cells. A: concentration-dependent stimulation of p38 (top curve) and c-Jun NH2-terminal kinase (JNK; bottom curve) phosphorylation by B. Cells were stimulated with B (10⁻¹¹–10⁻⁷ M), and the phosphorylation of p38 and JNK was determined by immunoblotting. Quantification of the p38 and JNK phosphorylation was determined by densitometric analysis of immunoblot. Data are means ± SE from 3 independent experiments. B: time course for the stimulation of p38 (top curve) and JNK (bottom curve) phosphorylation by B. Data are means ± SE from 3 independent experiments. C: effect of RU-38486 on B- and B-BSA-induced p38 and JNK phosphorylation. Cells were incubated with B (10⁻⁹ M), B-BSA (10⁻⁷ M), and BSA (10⁻⁷ M) for 15 min after pretreatment with GC nuclear receptor antagonist RU-38486 (10⁻⁶ M, 30 min) or no pretreatment. The phosphorylation of p38 (a, top) and JNK (b, top) was determined by immunoblot; a, bottom and b, bottom show total p38 and total JNK using phospho-independent MAPK antibodies. D: effect of genistein on B-stimulated p38 and JNK phosphorylation. Cells were stimulated with B (1 nM) and nerve growth factor (NGF; 100 ng/ml) for 15 min in the absence or presence of genistein (100 μM) for 15 min, and the phosphorylation of p38 (a, top) and JNK (b, top) was determined by immunoblot. E: effect of Go-6976 on B-stimulated p38 and JNK phosphorylation. Cells were stimulated with B (10⁻⁹ M) in the absence and presence of Go-6976 (10⁻⁷ M) for 15 min. Cells treated with PMA (10⁻⁶ M, 15 min) alone could also lead to the phosphorylation of p38 (a, top) and JNK (b, top). F: nuclear translocation of extracellular signal-related kinase (ERK)-1/2 MAPK in response to B, B-BSA, and NGF. Cells were either left untreated (control) or treated with BSA (5 nM), NGF (100 ng/ml), or B-BSA (100 nM) for 15 min. Cells were fixed and permeabilized, then incubated with primary antibody anti-ERK1/2 (top left), anti-active ERK1/2 MAPK antibody (control and bottom), and then FITC-conjugated antibody. Images were captured using a confocal microscope. Results are representative of 3 independent experiments. A–E are reproduced from Ref. 10 with permission. F is reproduced from Ref. 17 with permission.

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induced by BK, the \( \mathrm{Ca}^{2+} \)-free/\( \mathrm{Ca}^{2+} \)-reintroduction protocol was adopted in the experiment. The result showed that the \( \mathrm{Ca}^{2+} \) influx induced by BK could be rapidly inhibited by B, but intracellular \( \mathrm{Ca}^{2+} \) release was not affected (5).

Using \( \Delta \mathrm{[Ca}^{2+}] \), as an indicator, the involvement of the G protein-PKC pathway in the rapid, nongenomic action of GC in PC12 cells has been analyzed in detail. High K\(^+\) depolarizes the cell membrane, then opens the voltage-dependent \( \mathrm{Ca}^{2+} \) channel, causing \( \mathrm{Ca}^{2+} \) influx into cells. The inhibitory effects of B on the \( \Delta \mathrm{[Ca}^{2+}] \), induced by high K\(^+\) in PC12 cells is related to the time of B preincubation. The inhibitory effect started at 3 min of preincubation, reached maximum at 5 min, and was not obvious at 25 min (12). A G protein inhibitor, either pertussis toxin or GDP\( \beta \)S, significantly reduced the inhibitory effect of B and B-BSA. It is interesting to note that phorbol 12-myristate 13-acetate (PMA), a stimulator of PKC, could inhibit \( \Delta \mathrm{[Ca}^{2+}] \) induced by high K\(^+\) as well. On the other hand, inhibitors of PKC, chelerythrine chloride (CHE) and bisindolylmaleide, could significantly antagonize the inhibitory effect of GC on \( \Delta \mathrm{[Ca}^{2+}] \). This inhibition of nicotine-induced \( \Delta \mathrm{[Ca}^{2+}] \), by B and B-BSA could also be mimicked by the PKC activator PMA and reversed by PKC inhibitors CHE and G\( \delta \)-6976 (16). The results strongly suggested that the G protein-PKC system is involved in GC's rapid action in PC12 cells.

Although pharmacological experiments clearly demonstrated the involvement of the G protein-PKC pathway in GC's rapid action, direct measurement of the PKC activity in vitro was desirable. We determined PKC activity in the membrane fractions using the SignaTECT PKC assay system from Promega. The results showed that B could activate PKC activity and the PKC inhibitor CHE could completely block the activation of PKC. The time course showed that the PKC activity was kept at a high level from 5 to 15 min of B's action. The dose-response curve was bell-shaped, with a maximal activation at \( 10^{-9} \) M at 37°C but at \( 10^{-5} \) M at 25°C. This finding explained why the inhibitory effect was most efficient after 5 min preincubation with B before the addition of nicotine and high K\(^+\). It has also been shown that B-BSA can directly activate the PKC activity (Fig. 2).

In PC12 cells, cAMP levels and PKA activities were measured after treatment with B and B-BSA. It has been shown that B and B-BSA had no effect on either the resting, vasoactive intestinal peptide-stimulated, or forskolin-stimulated cAMP level and PKA activities (16).

In cerebral cortex synaptosomes and SK-N-SH cells, GDP\( \beta \)S significantly blocked the augmenting effect of B on uptake of glutamate. An activator of PKA, dibutryl cAMP, had no effect on the uptake of glutamate in synaptosomes, SK-N-SH cells, or BT-325 cells. The results indicated that PKA could not be involved in the action of GC on the uptake of glutamate. On the other hand, the involvement of PKC was uncertain (18).

A new model of GC's rapid nongenomic action

On the basis of previous studies of the rapid, nongenomic actions of GC and the protein kinase pathways, it is clear that multiple signal transduction pathways are involved in the rapid, nongenomic effects of GC in neural cells, and a new model of GC's rapid nongenomic action has been proposed (Fig. 4A). In the model, GC first acts on the putative membrane receptor and then via the PKA and pertussis toxin-sensitive G protein-PKC pathways to induce a variety of intracellular responses in neurons, glia, and pituitary cells. It should be emphasized that other pathways have been indicated (5) and new pathways might be disclosed. The pleiotropism in steroid's rapid action is still a big challenge for the future.

Possible genomic sequel of nongenomic action of GCs

It is well known that an elevation of cAMP levels in the cell may result in the activation of transcription through cAMP response element binding proteins. This implies the possibility of genomic activation through nongenomic activation by GCs. However, recent data showed that the activation of mitogen-activated protein kinases (MAPKs) is also involved in the rapid action of GC. Using Western immunoblot and protein kinase activity assays, for the first time we showed that B can induce a rapid activation of extracellular signal-related kinases (ERK)-1/2, p38, and c-Jun NH\( _2 \)-terminal kinase (JNK) MAPKs in PC12 cells. The dose-response curve was bell shaped, with the maximal activation at \( 10^{-9} \) M in 15 min (Fig. 3). Activation of ERK1/2, p38, and JNK by B was apparently not mediated by the classic cytosolic steroid receptors, because B-BSA can...
induce the phosphorylation of these MAPKs but the antagonist of GC nuclear receptor (RU-38486) cannot block the phosphorylation of ERK1/2, p38, or JNK induced by B. Phosphorylation of ERK1/2, p38, or JNK induced by B was not affected by a tyrosine kinase inhibitor (genistein), suggesting that the pathway does not involve the tyrosine kinase activity. On the other hand, PKC activator PMA can activate and PKC inhibitor Gö-6976 can block the activation of ERK1/2, p38, and JNK induced by B. Together, these data clearly demonstrated that B might act via putative membrane receptor and rapidly activate ERK1/2, p38, and JNK through PKC in PC12 cells, which might be mediated via cRaf-1. The results from immunofluorescence staining also revealed that the activated ERK1/2 was translocated from cytoplasm to nucleus in PC12 cells in 15 min (Fig. 3).

It is generally accepted that MAPK is in the downstream of receptor tyrosine kinase activation. This serine/threonine kinase, which can translocate into the cell nucleus, phosphorylates many different proteins, including transcription factors that regulate expression of important cell cycle and differentiation-specific proteins. Therefore, it is reasonable to postulate that there is a signaling pathway by which GC might affect the expression of genes that is not activated via GC receptor activation, i.e., the classic genomic pathway, but is responsive to factors acting through other pathways: PKA-cAMP response element binding protein or PKC-MAPK pathways. In short, GC can not only enhance the gene expression regulated by the nuclear receptors, i.e., the classic genomic mechanism, but can also cause a new pattern of gene expression that is the sequel of nongenomic activation, i.e., the nongenomic-genomic mechanism (Fig. 4B).

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References


