Genomic and non-genomic effects of steroïds on neuronal activity

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The discovery of intracellular steroïd hormone receptors in the 1960s presaged the present era of investigation of DNA-binding, gene regulatory proteins, of which the steroïd/thyroïd hormone receptor superfamily is an excellent example. It is therefore not surprising that steroïds are identified in many quarters of modern biology as agents that regulate gene expression. Another area of steroïd action is related to their lipophilic character and their effect on cell surface events. For example, the efficacy of synthetic and natural relatives of progestational steroïds as anesthetics has been known for many years.

However, the relatively high concentrations of steroïds needed to produce such effects, as well as ignorance about a molecular mechanism of action, relegated such apparent membrane actions to the back burner while pharmacologists pursued more precisely defined molecular interactions.

During the past few years, the momentum has shifted with the discovery of molecular interactions of steroïds with membrane events. At the same time there has been a reinvestigation of a host of rapid and possibly membrane-based actions of natural and synthetic steroïds on a variety of tissues. The effects o fsteroïds on excitable tissues and neurosecretory processes are most prominent, but there are also physiologically relevant actions of progestins on the maturation of spermatozoa and of oocytes. A picture is emerging that shows that membrane and intracellular actions of steroïds work, sometimes in concert, to produce shortand long-term modulation of cellular events, particularly within the nervous system. In fact, there has been sufficient interest in this topic for it to be treated in depth recently at a ClBA Foundation Symposium .

Steroïds and the GABAA receptor

The GABAA/benzodiazepine receptor Cl channel complex is a member of the same ligand-gated ion channel superfamily as the nicotinic acetylcholine receptor/ sodium channel complex, and may exist in a number of forms determined by its subunit composition. The receptor contains several functional domains, including a Cl- channel, a GABA recognition domain and a benzodiazepine recognition domain. Certain steroïds positively modulate GABA-induced Cl- flux in a manner that resembles that of the barbiturates, although the steroïd modulatory site is believed to differ from that of the barbiturates. In general, the steroïds that most effectively modulate Cl- flux are A-ring reduced, or pregnane steroïd. There are two known natural steroïd metabolites that are effective modulators of Cl- flux: allopregnanolone 5alpha-pregnan-3alphaOH-20-one or 3alpha-OH-DHP, which is derived from progesterone; and 5alpha-pregnan-3alpha,21diol-20-one (THDOC), which is derived from desoxycorticosterone. Both produce their effects on Cl- flux with IC50 values in the 10-50 nm range (these steroïd concentrations are only effective when GABA is also present). Both metabolites have been identified in blood and brain tissue and show increased concentrations within minutes after stress. THDOC levels rise during stress from <1 nm to reach concentrations -10 nm, which are within the sensitive range of the receptor. Moreover, enzymes in the brain generate these metabolites from the parent steroïds, and local concentrations of the metabolites may reach high nanomolar concentrations because of the contribution from this local production. The progesterone metabolite 3alpha-OH-DHP is also found in females and its levels in blood vary during reproductive cycles. Furthermore, anxiolytic effects of at least one of these metabolites, THDOC, have been detected after administration to rodents, suggesting that stressinduced elevations or fluctuations during the reproductive cycles might produce behavioral effects. This, however, remains to be established. In addition to their anxiolytic actions, these steroïds also have

antiepileptic activity.

Facilitation of the inhibitory effects of GABAA receptor activation by steroïds appears to be a likely mechanism for the anesthetic actions of many of these same steroïds, although perturbations of the membrane bilayer may also play a role. In order to define more precisely the role of the protein structure of the GABAA receptor complex in the actions of steroïds and other modulators, these receptors have been expressed in cells transfected with DNA containing sequences for the various subunit types. Expression of alpha and beta subunits results in receptors that respond to GABA as well as bicuculline and barbiturates; however, benzodiazepine sensitivity is reported to be missing in alpha-beta chimerae and conferred by the inclusion of DNA for ∂ subunits in the expression system. steroïd sensitivity apparent when beta alone, or alpha plus beta or alpha plus beta plus ∂ subunit DNA is expressed Thus the receptor protein itself is a key factor in determining steroïd sensitivity the GABAA receptor. There some preliminary evidence that different types of alpha subunit confer differing degrees of steroïd sensitivity on the resultant GABA receptor complex when co-expressed with beta and ∂ subunit DNA.

Progestational steroïds and Ca 2+

Oocytes and spermatozoa exist in fluids that contain reproductive hormones, and certain aspects their maturation are regulated the progestational steroïds. Studies on the frog oocyte have revealed a mechanism of action of progesterone at the oocyte surface that causes increased levels of Ca2+ within the egg cytoplasin. These effects of progesterone are rapid, with onset latencies of 40-60s; they last for 5-6 min. This Ca2+ mobilization is involved the meiotic maturation of th oocyte.

The acrosome reaction maturational event in spermatozoa that involves a proterone stimulated influx of cellular Ca2+ similar to the events occurring in the oocyte. Progesterone itself is very effective in producing these effects, and structure-activity profile of other steroïds appears to differ from that of the GABAA receptor activation described above, although many key steroïds have not be tested on both systems. The effects of progesterone occur within 30-60 s and are due influx of Ca+2+ from the extracellular space, since they are blocked both by the chelator EGTA and by the Ca 2+ channel antagonist lanthanum ions. The effect of progestins on Ca2+ levels inside cells is relevant to the actions of these steroïds on neurotransmitter and neurohormone release and such effects are summarized in Table 1. Transmitter release depends on Ca2+ and the modulation of Ca 2+ availability within the cell may represent a general mechanisrn of action of such steroïds at the cell surface.

Other rapid effects of steroïds

The specific interactions of steroïds with putative membrane receptor sites related to the GABAA receptor and to Ca` flux have prompted a re-examination of some old phenomena as well as new investigations of nongenomic effects of steroïds. As shown in Table I, there is an impressive array of effects, some of which may have physiological relevance, while others may not. The higher the steroïd concentrations that are required for effect, the less likely they are to be achieved in vivo. For example, the interference by 2-hydroxyestradiol of dopamine and alpha1-adrenoceptor binding involves extremely high steroïd concentrations that are unlikely to be achieved in vivo. Another consideration is whether the effect itself occurs in vivo. The actions of 3alpha OH-DHP and THDOC on the GABAA receptor, as well as the actions of progesterone on oocyte and sperm maturation, are likely candidates.

Steroïds have a number of rapid effects on neurosecretion . These actions occur at concentrations that are physiologically quite reasonable, and they are rapid in onset, occurring as rapidly as measurements of release can be made after adding them to the fluid bathing the cells. A case can be made for the actions of progestins on luteinizing hormone releasing hormone (LHRH) and dopamine release occurring in vivo.

Table 1 also notes extremely rapid effects of steroïds on electrical activity of nerve cells, occurring when applied locally. Methods of application include iontophoresis and pressure ejection, where experiments are carried out in vivo, and application to the bath for in vitro preparations. The onset latencies of these effects are all within seconds of application, which excludes any type of genomic action. Structure-activity studies in at least one case indicate a considerable degree of steroïd specificity, which argues for a receptor or recognition site at or close to the cell surface.

In pursuit of membrane receptors

One of the most elusive goals has been to obtain evidence with radiolabeled steroïds for the existence of putative membrane receptor sites. A number of studies showing membrane binding sites for steroïds are summarized in Table 1. A study by Towle and Sze employing a centrifugation assay, produced suggestive evidence for the existence of brain membrane binding sites for glucocorticoids, androgens, progestins and estrogens. A more recent study, using a filtration assay, has demonstrated high affinity membrane-associated receptors for estrogens in the rat pituitary gland.

One of the reasons for the elusiveness of membrane sites for steroïds may be their low abundance. Availability of higher specific radioactivity 125 I-labeled progestin has now allowed the successful identification of binding sites in brain membranes which have some of the specificity expected of the sites that regulate neurosecretion. The adsorption of steroïds by membranes is another problem; this means that nonspecific binding is inevitably very high and makes extensive studies of membrane binding extremely difficult.

In two instances, steroïds have been immobilized on macromolecules in order to demonstrate cell surface actions and receptors. In one series of experiments, progestins conjugated to serum albumin were used both for the 125I labeled progestin binding studies to brain membranes and to demonstrate the effects of steroïds on neurosecretory processes. In another study, estradiol conjugated to nylon fibers was used as an affinity column to isolate cells with membrane estrogen receptors. The conjugation of steroïds to macromolecular supports thus shows great promise in identifying membrane steroïd receptors and in isolating receptor-containing cells, as well as providing more definitive evidence for membrane steroïd actions mediating cellular processes.

Genomic actions of steroïds

In excitable tissues such as brain, long-term signaling by circulating hormones via genomic mechanisms plays an important role in shaping cell structure and function.

In the nervous system, the effects of steroïds are usually confined to particular groups of cells that contain intracellular steroïd receptors. The effects produced through these receptors range from the induction of key enzymes of neurotransmitter metabolism and neurotransmitter receptors to induction of synaptic and dendritic structure. Most of these changes are reversible ones, and some have been shown to vary during natural endocrine cycles such as the estrous cycle of the female rat.

The effects of estrogens are among the best studied. In basal forebrain cholinergic cells, for example, estradiol induces the enzyme choline acetyltransferase which is the key enzyme for acetyl choline biosynthesis. In ventromedial hypothalamus, estradiol induces synthesis of receptors for the neuropeptide oxytocin and also induces oxytocin formation in the cells that appear to innervate these receptors; as will be discussed below, these events may have importance for estrogen induction of female sexual behavior. Estradiol also induces synthesis of progesterone receptors in a number of brain regions, as w as in the reproductive tract and pituitary, and this induction is important for induction of sexual behavior. Longterm estrogen induced increases in 5-HT1 receptors and GABAA receptors also occur in certain brain regions, and increases in mRNA for enkephalin in the ventromedial nuclei of hypothalamus have been observed. All of these effects

take many hours or days to appear, and occur only in brain areas with intracellular estrogen receptors. Thus they appear to be genomic effects. Perhaps the most surprising finding has been that estradiol induces spines on dendrites neurons within the hypothalamus and hippocampus of the female rat; since spines are occupied by synapses, this finding strongly suggests that the hormone is regulating synaptogenesis; electron microscope data support this suggestion . Even more prising is that spine densities these same dendrites rise and throughout the four-day estrous cycle of the female rat.

Other steroïds also produce long-lasting and apparently genomic effects on neural tissue. In addition to regulating the neuropeptide systems that govern ACTH release from the pituitary gland, glucocorticoids regulate a number of enzymes and structural proteins throughout the brain as well as having long-term effects on neuronal survival and destruction.

Another type of long-lasting steroïd effect that alters tissue excitability is illustrated by a recent study of the action of androgen in muscle. Androgens act on myotubes from frog muscles, which contain intracellular androgen receptors, to exert a long-term increase of acetylcholine-activated single channel conductances, suggesting that the steroïd induces a substance that modulates channel behavior in the acetylcholine receptor. This study emphasizes the importance of careful analysis of how actions of a steroïd produce a membrane effect, to distinguish between genomic and nongenomicic mechanisms.

The most obvious way of distinguishing these mechanisms is on the basis of time-course, with rapid onset of effects being nongenomic and possibly membrane mediated, and effects that are slower in onset being genomic. The problern in making a distinction lies in the time range of minutes, where rapid genomic or long-lasting membrane effects might be involved. To resolve such cases, inhibitors of protein or RNA synthesis may be useful, along with other means of isolating the membrane from the rest of the cell, such as patch clamping.

Genomic and non-genomic effects in concert

Figure 3 summarizes some of the actions of progesterone and its metabolites, at the membrane level and via the genorne, emphasizing the fact that metabolism of the steroïd by enzymes in neurons, or on or in glial cells, may be a regulatory mechanism for generating the steroïd derivatives that have effects on different membrane receptors. The gene products that are regulated by progesterone acting via intracellular receptors are not identified as yet. However, their apparent importance for the modulatory effects of progesterone on reproductive behavior has been recognized. The scheme shown in Fig. 3 is also applicable to other steroïds such as those from the adrenal cortex, where metabolism generates some membrane-active steroïds and where genomic actions of the parent steroïd are also known.

Interaction and cooperation could theoretically occur between genomic and non-genomic steroïd responses. Indeed,effects such as those of the progestins on responses of neurons to glutamate and GABA are independent of prior estrogen priming. However, some membrane effects of iontophoretically applied estradiol on neural activity are influenced by the stage of the estrous cycle. This implies a dependence on the actions of ovarian hormones, possibly via genomic mechanisms.

We have recently found that estradiol induces oxytocin receptors in the ventromedial hypothalamus of the female rat and that at least some of these induced receptors, located in the caudal ventromedial nuclei, will then respond rapidly to the administration of progesterone. Within less than an hour, progesterone causes an apparent spread of the oxytocin receptor field in a direction lateral to the cell bodies where the receptors are induced. It appears that this spread may be occurring along the dendrites of the ventromedial nucleus neurons which project laterally. Much

to our surprise, progesterone was able to produce the same effect in vitro in previously freeze-dried sections prepared for binding of the oxytocin receptor ligand. The fact that this action occurs in vitro at 100 nm progesterone and appears to be the same as the effect of physiological doses in vivo argues strongly that this is a physiologically relevant membrane effect of the steroïd. It may represent a rapid movement of receptors along the dendrites or it may be an activation of low-affinity receptors to a high-affinity form capable of binding the ligand. As far as we know, the steroïd specificity favors progesterone over estradiol cholesterol and some of the progestin metabolites that affect the GABAA receptor, but further studies are required. Whichever mechanism is involved, the result of this action of progesterone appears causally linked to the induction of sexual behavior in the female rat, based upon behavioral and anatomical studies.

Steroïd hormones have long been regarded as acting on cells via totally different sites and mechanisms of action from those of neurotransmitters. Neurotransmitters, as well as hormones that act on the surface of cells, were traditionally thought to influence only short-term responses at the cell membrane. The discovery of increasing numbers of second messengers and processes linked to them has led to the realization that they affect many intracellular processes, including changes in gene expression. Now, with the recognition of the actions of steroïds on membranes as described in this article, the distinction has broken down further. That is, the actions of steroïds appear to involve the cell membrane as well as the genome, and (although it has not been demonstrated) it is even conceivable that actions of steroïds at the cell surface might be able to trigger changes in gene expression indirectly.

We need to know much more about membrane effects and the steroïds that cause them before we can fully appreciate the connections between their actions at the membrane and on the genome. First, it is important to know whether there are unifying processes that underlie at least some of the membrane effects summarized in Table 1. For example, how many of these actions may be explained by steroïd-induced alterations in intracellular Ca2+ ? Furthermore, is the action of steroïd on the GABAA receptor an isolated example, or do other complex receptors such as those for acetylcholine or NMDA also contain steroïd recognition sites? Secondly, it is essential to reexamine the extensive work on steroïd metabolism in brain to see how many natural metabolites of steroïds have membrane activity, as the A-ring reduced metabolites of progesterone and adrenal steroïds have on the GABAA receptor. Moreover, the known membrane effects of steroïds must be compared across the same group of steroïds in corder to understand fully similarities and differences in structure-activity profiles. Thirdly, it is important to find out whether all membrane associated receptor sites can be ascribed to protein structures in the membrane, or whether there is a separate class of 'recognition' sites defined largely by the cornposition of menbrane lipids. Finally, development of new psychoactive drugs may benefit from considering the membrane-active steroïds either themselves or as starting points for the creation of new synthetic compounds. The diversity of structure-activity profiles already evident in the case of the GABAA receptor makes this area a fertile one for future research.