Pharmacology Biochemistry and Behavior, Vol. 64, No. 2, pp. 311–317, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00056-8

Hormones of the Hypothalamo–Pituitary–Gonadal Axis in Drug Discrimination Learning

RENÉ DE BEUN

Department of Behavioral Pharmacology, Novo Nordisk A/S, Måløv, DK-2760, Denmark

DE BEUN, R. *Hormones of the hypothalamo–pituitary–gonadal axis in drug discrimination learning.* PHARMACOL BIOCHEM BEHAV **64**(2) 311–317, 1999.—More than 30 years ago, T-maze studies with progesterone indicated that sex hormones have the potential to act as a discriminative stimulus in rats. Despite these early positive findings, the interest in discriminative stimulus properties of sex hormones remained low; few studies were dedicated to the investigation of discriminative stimulus properties of hypothalamo–pituitary–gonadal axis hormones (i.e., LHRH, LH/FSH, sex steroids). Nevertheless, the few studies that were published showed some interesting, and often sex-dependent results. Applying various methodologies (T-, or Y-maze, two-lever drug discrimination, taste aversion procedures), it was found that not only progesterone but also the two other principal sex steroids estradiol and testosterone can serve as discriminative stimuli in rodents. In addition to these gonadal hormones, the hypothalamic peptide LHRH (having a key role in the neuroendocrine regulation of steroid release from the gonads) appears to generate discriminative stimulus properties. Interestingly, recent (but preliminary) studies in postmenopausal women suggest that estradiol (and possibly progesterone) may also function as a discriminative stimulus in human subjects. © 1999 Elsevier Science Inc.

Drug discrimination Gonadal hormones Steroids Estradiol Testosterone Progesterone LHRH Sex differences

THE drug discrimination (DD) methodology has become one of the major techniques for the investigation of behavioral and neuropharmacological effects of compounds, and currently there is little doubt that this paradigm is a most valuable tool in drug discovery. Since the early 1970s, the DD field has grown very rapidly, and by now the "Comprehensive Bibliography of Drug Discrimination Research" (27) contains a list of more than 2,500 references. Despite the popularity of the DD procedure in behavioral pharmacology research, some potentially interesting substances have received very little attention. One class of compounds that has been neglected almost completely in DD studies is the sex hormones. Studies aimed at revealing discriminative stimulus properties of the gonadal steroid hormones testosterone, estradiol, and progesterone are scarce. Moreover, the number of studies directed at other hormones involved in the hypothalamo–pituitary–gonadal axis (LHRH, LH, FSH) is closed to zero. The present article gives a summary of the findings with sex hormones in discrimination learning. The current status of these compounds within this field of research and possible directions for future investigations are discussed.

DISCRIMINATIVE STIMULUS PROPERTIES OF PROGESTERONE

Progesterone was the first sex hormone that was investigated for generating discriminative stimulus properties. In the late 1960s, this steroid was submitted to a T-maze procedure in rats, together with "Viadril" (hydroxidione sodium), a chemical structure closely resembling the structure of progesterone (26). The aim of the studies was to show that compounds that are normally produced endogenously could result in that the investigators called "state-dependent learning." The rationale for choosing progesterone was that this hormone was known to show hypnotic properties at high doses, and hypnotics had previously been found to be effective in supporting state-dependent learning. The initial studies were done with Viadril (a compound with strong hypnotic properties), in intact male hooded rats, followed by progesterone studies in ovariectomized (OVX) female hooded rats. The animals were trained to escape electric shock delivered in the starting arm by entering one of the two other arms. Dependent on the rat's drug-state, the left or right arm of the T-maze was "safe" (nonshocked arm). The injection-session interval was variable, and ranged between 15 and 30 min because the animals were only tested "as soon as signs of sedation (ataxia, drowsiness) were evident." In this procedure, male and female rats relatively quickly learned to discriminate Viadril (25 mg/kg IP) and progesterone (100 mg/kg IP), respectively, from saline. The stimulus effects were robust and reached for both compounds a level of 80% correct responses, with apparently no differences in the shape of the acquisition curves. It cannot be inferred from the data whether performance level during progesterone training was equal under drug and nondrug conditions. Although the amount of data obtained in

the progesterone studies was somewhat limited (no dose–response studies, no crossgeneralization tests, only female rats used), these early T-maze experiments were the first proof that sex hormones have the potential to function as discriminative stimuli. Progesterone was convincingly effective as a training cue, in this case probably based on hypnotic properties. At a time point that DD methods were not yet widespread, the investigators interpreted the progesterone effects as state-dependent learning, and not as DD learning. But after DD paradigms saw a rapid development in the early 1970s, and given the specific design of the T-maze studies in question, it now seems more appropriate to describe the reported effects in DD terms.

Although the T-maze results looked promising, it took 2 decades before these studies were followed by a two-lever DD study with progesterone (14). Meanwhile, attention was mainly focused on (true) state-dependent learning effects of the ovarian steroids progesterone and estradiol. That is, state-dependent learning based on different phases of the estrous cycle in female rodents was investigated, producing somewhat ambiguous results [e.g., (9,12,15,20)]. Although state-dependent learning and DD can be considered related phenomena, it has become clear that the learning principles underlying these paradigms can be dissociated [e.g., (1,17,22)]. It was not before the late 1980s that the discriminative stimulus effects of progesterone, as assessed in the T-maze, were confirmed using a standard two-lever paradigm (14). In this study, both intact male and OVX female Wistar rats were trained to discriminate pentobarbital (12 mg/kg IP, −15 min) from saline. In subsequent crossgeneralization tests with different doses of progesterone (10, 20, 40, and 80 mg/kg IP, −30 min), it was shown that progesterone dose dependently substituted for pentobarbital in OVX female rats. Partial generalization from pentobarbital to progesterone was observed after the 40-mg/kg dose (approximately 50% drug-lever responses), whereas full generalization was seen after the 80-mg/kg dose (close to 90% drug-lever responses). Interestingly, the stimulus effects of progesterone were found to be sex dependent. Pentobarbital did not generalize to progesterone in the gonadally intact males, although no sex differences were observed in the acquisition of pentobarbital discrimination, or in the generalization gradient of pentobarbital itself. It was concluded that progesterone can exert discriminative stimulus control over behavior, based on properties shared with central sedatives (i.e., based on properties shared with a representative barbiturate). With respect to the apparent sex difference, the use of intact male vs. OVX females may not have been the best possible design to draw firm conclusions. Testosterone is converted into estradiol by the enzyme aromatase in certain parts of the CNS and, different from the females, the presence of both testosterone and estradiol may have been a confounding factor in the males. The use of gonadectomized (GDX) males might have been a better option, excluding that progesterone stimulus effects may be influenced by the testosterone/estradiol background. Thus, the observed sex difference could be based on interactions with so-called activational effects of testosterone or estradiol in the males, rather than being a consequence of the process of sexual differentiation of the CNS, which is dependent on organizational effects of sex steroids around birth (10).

In a recent study conducted in our labs (unpublished data, 1997), the reported substitution of progesterone for pentobarbital could only be partially reproduced in a similar two-lever DD procedure. A pentobarbital cue (10 mg/kg IP, −15 min) was established in GDX Wistar rats of both sexes, followed by generalization tests with progesterone (20, 40, and 80 mg/kg SC, −30 min). In line with the results of the above-mentioned study, conducted in intact males (14), no substitution of progesterone for pentobarbital was noted in the GDX males. This indicates that the observed lack of stimulus substitution of progesterone for pentobarbital in males was independent of the gonadal status of the animals. However, the full generalization from pentobarbital to progesterone in OVX females could not be reproduced. Only partial substitution was observed, with approximately 50% drug-lever responses after 80 mg/kg of progesterone. Although there were some procedural differences between the two studies (12 vs. 10 mg/kg training dose of pentobarbital, IP vs. SC route of administration for progesterone), it remains unclear what caused this discrepancy in results. It seems unlikely that the animals in the second study were less sensitive to the progesterone effects, as clear signs of sedation were noted after treatment with the 80-mg/kg dose. Furthermore, latency time for making the first lever press was in this group of animals considerably longer than in the vehicle-treated controls (because the generalization sessions were nonreinforced, response rate after progesterone was not measured).

The most comprehensive study focusing on discriminative stimulus properties of ovarian hormones was conducted in OVX female Sprague–Dawley rats using a Y-maze (11). Similar to the previously described T-maze procedure with progesterone (26), the animals were trained to terminate electric shock delivered in the starting arm by entering one of the other two arms. Dependent on the rat's drug state, entry of the left or the right arm of the Y-maze terminated a mild foot shock. In this procedure, progesterone (0.5, 2, 4, and 8 mg/kg), its active metabolite dihydroprogesterone (4 mg/kg) and estradiol benzoate (6.4 μg/kg) were investigated for their ability to support discrimination training. All compounds were injected IP, with an injection-session interval of 4 h. In this way, a dose-dependent progesterone cue was established. The animals successfully discriminated progesterone from oil when the training dose was 4 or 8 mg/kg (approximately 65 and 75% correct responses, respectively). The lower doses of progesterone were ineffective, the percentage of correct responses being similar as in a vehicle–vehicle trained group (i.e., around 30%). In addition, the animals could also be successfully trained to discriminate one of the main metabolites of progesterone, dihydroprogesterone, from oil (about 75% correct responses, with a 4-mg/kg dose as the training cue), indicating that the stimulus effects of progesterone could actually be based on effects of this metabolite. As was the case for the T-maze studies with progesterone (26), it cannot be inferred from the data whether performance level was equal under drug and nondrug conditions after progesterone and dihydroprogesterone discrimination training. Furthermore, a potential problem with these studies is that an alternating drug/nondrug treatment schedule was used rather than a (semi)random schedule. It cannot be excluded that the behavior of the animals was (partly) guided by the treatment schedule per se, and not (only) by discriminative stimulus properties of progesterone or its metabolite. The finding that the control animals performed significantly worse in selecting the correct arm (about 30% correct selections) than can be expected on a random base (50%) suggests that some "schedule-learning" occurred, and it may have been the case that treatment with progesterone and dihydroprogesterone interfered with this learning behavior. Interestingly, in a subsequent drug-vs.-drug procedure, the animals were able to learn to discriminate progesterone (4 mg/kg) from its metabolite dihydroprogester-

one (4 mg/kg), suggesting that the stimulus effects of these steroids are dissimilar (a level of about 60% correct lever responses was reached vs. about 30% correct responses in the vehicle/vehicle control group). However, because equal doses were used for the progesterone and the dihydroprogesterone, these latter results could still be interpreted as the animals being able to discriminate different levels of the metabolite (which is presumably considerably less in the progesterone-treated group than in the metabolite-treated group). In an additional drug-vs-drug experiment, it was shown that progesterone (4 mg/kg) could also be discriminated from the other principal ovarian hormone, estradiol (6.4 μg/kg of estradiol benzoate), with a performance level of almost 80% correct arm selections (this dose of estradiol benzoate was in itself sufficient to serve as a cue in these Y-maze studies; see estradiol section). These data suggest that both progesterone and estradiol generate discriminative stimulus properties in OVX female rats, but with a different quality. To control for a possible involvement of adrenal steroids (sex and stress hormones), the investigators repeated the discrimination studies with progesterone (4 mg/kg) vs. oil, and progesterone (4 mg/kg) vs. estradiol benzoate (6.4 μg/kg), in both OVX and adrenalectomized females. Although the adrenalectomy did, to a certain extent, impair the discrimination learning in both groups (with performance levels going down to about 70 and 60%, respectively) compared to females that were only OVX, the results nevertheless indicated that the action of adrenal steroids was not critical for the observed discriminative stimulus effects of progesterone (and estradiol).

Recently, some preliminary results were published of DD studies with progesterone in human subjects (25). In postmenopausal women, progesterone (micronized, 200 mg PO) could not be reliably discriminated from placebo. Out of six women, only one was able to identify the progesterone as being "different" from placebo. These studies were only a first attempt to reveal discriminative stimulus properties of progesterone in human subjects, and the apparent lack of effect of this steroid may have to do with many variables that were not controlled for. For example, it is not clear whether the women were on any kind of hormone replacement therapy in this study. Furthermore, variables like dose and pretreatment time were not subject of investigation.

DISCRIMINATIVE STIMULUS PROPERTIES OF ESTRADIOL

In the 1980s, estradiol was shown to act as an aversive stimulus in rodents, producing powerful conditioned taste aversion learning [CTA; (13,18,24)]. Despite this, and although positive results were obtained with progesterone in DD studies (14,26), it was not before the early 1990s that the first publication on discriminative stimulus properties of estradiol appeared (3). In this study, GDX Wistar rats of both sexes were trained to discriminate 17 β-estradiol (50 μg/kg SC, with an injection-session interval of 1 h) from oil using a discrimination paradigm based on taste aversion learning. After treatment with estradiol, consumption of a saccharin solution was followed by a sickness-inducing injection of lithium chloride (LiCl). After treatment with its vehicle, consumption of the saccharin was followed by a saline injection. Both males and females learned to differentially suppress their saccharin intake dependent on drug state. There were no sex differences in the discrimination acquisition curves, and both sexes finally reduced their fluid intake on estradiol sessions to a level of around 40% of the fluid intake seen on corresponding vehicle sessions, indicating that estradiol was effective as a cue in discrimination learning. To exclude the possibility that estradiol reduced saccharin intake per se, control groups were treated in the same way, but always received saline injections after the saccharin presentation. In these groups, no differences were noted in saccharin intake dependent on drug state. A subsequent generalization test in which different doses of 17 β-estradiol (0.4, 2, 10, 50, and 250 μg/kg SC, −1 h) were substituted for the training dose revealed dose-dependent discriminative stimulus effects of estradiol in both sexes, with overlapping dose–response curves. No crossgeneralization tests to other compounds were conducted, so nothing could be said about the quality of the estradiol cue. Furthermore, it was not investigated whether estradiol could also be established as a cue when it is not associated with the aversive consequences of the LiCl, but with the saline injections (and, vice versa, the vehicle being associated with the aversive stimuli produced by LiCl). That such "asymmetrical" stimulus properties can indeed be a potential problem in this procedure has been recognized in some other discriminative taste aversion studies [e.g., (28)].

In a recent attempt to replicate the positive results with estradiol as a cue in taste aversion DD learning, a standard two-lever DD study was conducted in our labs (unpublished data, 1998). GDX male and female Wistar rats were trained to discriminate 17 β-estradiol (100 μg/kg SC, with an injection-session interval of 1 h) from its vehicle. However, even after extensive training, discrimination learning was not observed in the males, or in the females. One reason for this failure to establish an estradiol cue may have to do with the chosen injection-session interval. If discriminative stimulus properties of estradiol are dependent on intracellular, nuclear estradiol receptor binding, involving transcriptional factors, it is obvious that a 1-h interval between injection and training session is too short (more reasonable would be about 4 h in this case). The relatively short interval used was based on the successful discriminative taste aversion studies with estradiol (3), and it remains unclear why there is such a discrepancy between the results of the two studies. Negative results of previous studies conducted in our lab (unpublished data, 1996) already indicated that it is difficult to establish an estradiol cue in a standard two-lever DD procedure. Under various training conditions, OVX female Wistar rats did not manage to discriminate 17 β-estradiol from vehicle. Different training doses, ranging from 10 μg/kg up to 300 μg/kg IP (the training dose was increased every time discrimination training remained unsuccessful at a given dose), and various injection-session intervals (between 15–180 min) did not result in any discrimination learning.

Shortly after it was found that estradiol can serve as a cue in a discriminative taste aversion procedure (3), another procedure based on taste aversion learning was used to compare the stimulus properties of estradiol with those of the other principal sex hormones progesterone and testosterone (7). In this study, gonadally intact female CD-1(ICR) mice were submitted to a crossfamiliarization CTA (CF-CTA) procedure, measuring preexposure effects of sex steroids (four daily injections prior to the conditioning session) on a 17 β-estradiol (50 μg/kg SC)-induced CTA towards a glucose solution. The CF-CTA procedure makes use of the phenomenon that exposing animals repeatedly to a drug that normally induces a CTA, prior to the association of this particular drug with a novel taste, reduces or abolishes the probability of developing a CTA. It is not only possible to prevent the formation of a CTA by preexposure to the same drug, but also by preexposure to a different drug. It is, therefore, argued by some inves-

tigators that preexposure CTA studies allow assessments of degrees of similarities of stimulus effects. Initially, it was shown that preexposure to estradiol itself (2, 10, and 50 μg/kg SC) dose dependently inhibits the development of a CTA by this hormone. The 50 μg dose completely blocked the taste aversion effect produced by the same dose of estradiol in the absence of the preexposure drug, indicating—as to be expected for identical drug conditions—full crossfamiliarization. In subsequent crossfamiliarization tests with progesterone (50, 100, and 200 μg/kg SC) and testosterone (0.25, 0.5, and 1 mg/kg SC), only partial crossfamiliarization was observed (after all three doses of progesterone, and after the highest dose of testosterone). It was concluded from these results that only a low degree of stimulus resemblance exists between estradiol and the other main sex steroids, progesterone and testosterone, although all of them have been shown to generate discriminative stimulus properties (3,6,11,14,26). An interesting finding of this study was that the "sickness-inducing" compounds LiCl (22 mg/kg SC) and apomorphine (0.1 and 0.2 mg/kg SC) did familiarize for estradiol (albeit only partially in the case of apomorphine). This suggests that the estradiol stimulus complex resembles, at least to a certain extent the stimulus complex produced by these aversive agents. A weaker point of this study is that the female mice were not OVX, leaving open the possibility that the data are somewhat blurred by interactions between the endogenously produced female sex hormones (at fluctuating levels) and the exogenously derived hormones. Furthermore, although several investigators have claimed that the CF-CTA method can be seen as an alternative for crossgeneralization tests in two-lever DD procedures, it is also clear that this method is less established than the traditional two-lever DD paradigm. It is still debated whether it is a "true" alternative for this latter tool, and there is a continued discussion on whether the same (discriminative) stimulus properties are measured in both procedures [e.g., (8)].

As mentioned already in the progesterone section, the most comprehensive study investigating discriminative stimulus properties of ovarian hormones was done in a Y-maze, in which OVX female Sprague–Dawley rats were trained to escape from electric shock delivered in the starting arm by entering one of the two other arms (11). Dependent on the rat's drug state, the left or the right arm of the Y-maze was the "safe" one. In this procedure, a dose of estradiol benzoate as low as 6.4 μg/kg IP was sufficient to serve as a cue to the animals when injected 4 h before the training sessions (almost 80% correct responses). Because no substitution tests were conducted to generalize from the training dose to other doses of estradiol benzoate, and because animals were only trained on a single dose, no statements could be made with respect to dose-dependent stimulus properties of this steroid. As was the case for the progesterone experiments in this study, it cannot be inferred from the data whether performance level was equal under drug and nondrug conditions. Crossgeneralization tests were not conducted, but a drug-vs.-drug procedure revealed that estradiol benzoate (6.4 μg/kg IP) could easily be discriminated from progesterone (4 mg/kg IP), with a performance level of close to 80% correct arm selections (the progesterone condition was previously shown to serve as a cue in these Y-maze studies; see progesterone section). Thus, although estradiol and progesterone both generated clear discriminative stimulus properties in OVX female rats, the cues appeared to be of dissimilar quality. The same conclusion was drawn from a previous CF-CTA study (7). The potential problem raised by the use of an alternating drug/nondrug treatment schedule has been discussed in the progesterone section and also may have played a role in the estradiol studies because "schedule learning and memory" phenomena were noticed again in the control group (only around 10% correct arm selections, instead of the expected 50% by chance).

In the same human studies in which it appeared almost impossible for postmenopausal women to discriminate progesterone from placebo (see progesterone section), more success was obtained with estradiol treatment (micronized, 2 mg PO) (25). Full discrimination was not observed, but 50% of the subjects reached the criterion set for discrimination. As mentioned before, it cannot be inferred from the data whether or not these women were on hormone replacement therapy. Furthermore, 2 mg of estradiol on eight sessions must be considered a high dose of this hormone, and it is also not clear from the data whether this produced some more general effects like nausea that could have signaled the presence of the compound.

DISCRIMINATIVE STIMULUS PROPERTIES OF TESTOSTERONE

Studies aimed at revealing discriminative stimulus properties of testosterone are scarce. Aside from the previously mentioned CF-CTA studies (see estradiol section), in which it was found that testosterone and estradiol generate dissimilar stimulus properties in female mice (7), only one further study has been published on this topic (6). In a discriminative taste aversion procedure, GDX male Wistar rats were trained to discriminate testosterone from oil (1 mg/kg SC, with an injection-session interval of 1 h). The animals learned to differentially suppress the intake of a saccharin solution dependent on the presence or absence of testosterone (consumption of the saccharin was followed by an injection of LiCl or saline, respectively). The final level of fluid intake on testosterone sessions was about 40% of the fluid intake on corresponding vehicle sessions. This indicates that testosterone could effectively be used as a cue in discrimination learning. A control group that was treated in the same way was included, but these animals always experienced the same "neutral" consequence of saccharin consumption, independent of drug state. This excluded the alternative explanation that testosterone simply reduced saccharin intake per se. A subsequent generalization test was different doses of testosterone (0.125, 0.25, 0.5, 1, and 2 mg/kg SC, −1 h) substituting for the training dose of this hormone revealed that the discriminative stimulus effects of testosterone were dose dependent in GDX male rats. Because only males were tested, nothing could be said about possible discriminative stimulus effects of testosterone in females. No crossgeneralization tests were conducted with, for example, other sex steroids. The already discussed potential problem of "asymmetrical" stimulus effects of compounds in this specific taste aversion procedure (see estradiol section), was not controlled for in this study either.

DISCRIMINATIVE STIMULUS PROPERTIES OF LUTEINIZING HORMONE (LH) AND FOLLICLE STIMULATING HORMONE (FSH)

No studies have been published investigating possible discriminative stimulus effects of the glycoproteins LH and FSH. The release of these gonadotropic hormones from the anterior pituitary is to a large extent regulated by hypothalamic LHRH and, aside from showing a variety of other endocrine effects, they stimulate the release of sex steroids from the go-

nads. Apparently, binding sites for LH and FSH are basically restricted to tissue of the testes (Leydig cells) and ovaries (theca, granulosa, luteal, and interstitial cells). The absence of CNS binding sites for the gonadotropic hormones, together with a lack of convincing evidence that these hormones possess any extraendocrine effects, makes it understandable that LH and FSH have not received attention in DD studies.

DISCRIMINATIVE STIMULUS PROPERTIES OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH)

Thus far, two studies have been dedicated to discriminative stimulus properties of LHRH, a centrally released decapeptide stimulating the release of the gonadotropins LH and FSH from the anterior pituitary. In the first study, conducted in the early 1990s (4), Wistar rats of both sexes were trained to discriminate LHRH (5 μg/kg IP) from saline in a two-lever DD procedure, using two different injection-session intervals of 15 and 45 min. The animals were GDX and received a Silastic SC testosterone implant to obtain adequate and stable negative feedback on the release of both the gonadotropic hormones and LHRH. When LHRH was injected 15 min prior to the training sessions, discrimination learning was not observed. Extending the injection-session interval to 45 min resulted in sex-dependent discrimination learning. The GDX males showed full discrimination of LHRH (approximately 85% correct lever responses), whereas the OVX females again failed to use this peptide as a cue. Subsequent generalization tests in the males, with different doses of LHRH (ranging from 62.5 ng/kg to 8 μg/kg IP) dose dependently substituted for the training dose. Partial substitution occurred at a dose as low as 250 ng/kg (close to 50% drug-lever responses); full substitution was observed with doses of 4 μg/kg or higher (≥80% drug-lever responses). In addition, the stimulus properties of LHRH were time dependent: In substitution tests with 5 μg/kg IP LHRH, where the injection-session interval was varied between 15 and 120 min, full generalization was found from the training interval to intervals between 45 and 60 min (about 80% drug-lever responses). Partial generalization was found to intervals between 75 and 105 min (50–55% drug-lever responses). No generalization was observed to intervals of 30 min or shorter, and to the 120 min interval. No crossgeneralization tests were conducted to compounds with well-characterized stimulus properties. Especially with LHRH, this would have been of great interest, as the LHRH systems in the CNS are known to interact with a variety of other peptide and neurotransmitter systems [e.g., (16)]. Vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), oxytocin, corticotrophin releasing hormone (CRH), β-endorphin, serotonin, adrenaline, noradrenaline, and dopamine all have been found to play a role in the activity of LHRH. In addition, it has been suggested that also α-melanocyte-stimulating hormone (α-MSH) and gamma aminobutyric acid (GABA) are involved in the modulation of LHRH activity.

Stimulus properties of LHRH were also investigated in a two-lever DD procedure after intracerebroventricular (ICV) administration (5). Initially, an LHRH cue was established after systemic administration of the peptide (5 μg/kg IP, −45 min) in GDX male Wistar rats (again provided with a Silastic SC testosterone implant to obtain adequate and stable negative feedback on the release of the gonadotropic hormones and LHRH). After successful training (reaching a level of just over 80% correct lever responses), the animals were equipped with a unilateral cannula in the left lateral ventricle, and were submitted to generalization tests with various ICV doses of LHRH (between 25 and 400 ng/rat, −40 min). Although full substitution was not observed, IP administered LHRH partially generalized to ICV administered LHRH. Within the dose range of 100–400 ng, ICV-administered LHRH produced approximately 50% drug-lever responses. In an additional generalization test with a dose of 200 ng/rat ICV LHRH, using different injection-session intervals (ranging between 10 and 40 min), full substitution for the IP LHRH still could not be obtained. Partial generalization was again observed, with the 30-min injection-session interval showing the highest degree of ICV LHRH substitution for IP LHRH (about 55% drug-lever responses). Nevertheless, these results indicated that centrally administered LHRH may serve as a dose- and time-dependent discriminative stimulus in male rats. No tests were conducted with a centrally administered LHRH receptor antagonist in combination with IP-injected LHRH. Blocking of the stimulus produced by systemically administered LHRH with an ICV administered antagonist would have strengthened the notion that the discriminative stimulus properties of LHRH are centrally mediated.

CURRENT STATUS AND FUTURE DIRECTIONS OF DRUG DISCRIMINATION RESEARCH WITH SEX HORMONES

It is clear that the number of studies on discriminative stimulus properties of sex hormones (gonadal steroids, gonadotropins, LHRH) is still very limited. DD studies with the gonadotropic hormones FHS and LH are lacking; only a couple of studies have been published on LHRH, and the steroids testosterone, progesterone, and estradiol were the subject of investigation in only a handful of studies. Furthermore, the scarce literature that is available is ambiguous, sometimes even confusing. It is difficult to get a clear picture about the discriminative stimulus properties of sex hormones when the findings are considered as a whole, because various experimental factors varied from one study to another. The most obvious drawback is that the number of different DD methods to reveal stimulus properties of sex hormones was almost as large as the number of studies actually conducted. Thus, only LHRH has been (successfully) used as a training drug in a standard two-lever food-reinforced DD procedure. None of the other sex hormones have been shown to be effective as a training stimulus in this, without doubt, most established and validated DD paradigm. Although progesterone was found to substitute for pentobarbital in crossgeneralization tests, evidence that this compound can also support the development of DD learning in a two-lever procedure is still lacking. Progesterone, estradiol, and testosterone all have been found to support discrimination learning, but in alternative DD procedures such as the T-maze (progesterone), the Y-maze (progesterone and estradiol), and the discriminative taste aversion technique (estradiol and testosterone). Not only were a variety of procedures used, also different species (rat or mouse) were used. For both species, the subjects were sometimes males, sometimes females, sometimes gonadally intact, sometimes GDX. And last but not least, different forms of the various hormones were investigated (e.g., 17 β-estradiol or estradiol benzoate). Nevertheless, the findings do indicate that all three principal sex steroids and LHRH have the potential to serve as a cue in DD learning, and it certainly seems worthwhile to investigate this potential in a more systematic and thorough way in future studies.

To confirm and strengthen the results obtained in earlier studies, the first challenge appears to be to establish an estradiol, a progesterone, as well as a testosterone cue in a two-

lever DD procedure. As mentioned, this still has not been achieved (or at least it has not been published). Thus far, a few attempts to establish these cues failed to do so. Preferably, these studies should be conducted in GDX rats of both sexes. Furthermore, if successful, systematic crossgeneralization studies should be done to characterize the stimulus complex of each of the sex steroids (and of LHRH). These substitution tests should go a bit further than only generalization tests to other sex hormones or active metabolites of these steroids (notwithstanding the fact that this is in itself also of interest). In addition, in the case that sex hormone cues are successfully established in two-lever DD procedures, antagonism studies would confirm mechanism of steroid action. Selective and potent receptor antagonists are available for progesterone, estradiol, testosterone, and LHRH.

With respect to estradiol, the recently discovered ERβ receptor may stimulate new research on discriminative stimulus properties of this hormone. The finding that there are at least two subtypes of the estradiol receptor (not only the "classical" estrogen receptor protein, which is now referred to as the ERα receptor) opens new possibilities for characterizing stimulus properties of estrogens on the level of receptor subtypes. This is of interest because it has been reported that the ERα and ERβ receptors are differentially distributed in different (brain) tissues [e.g., (2,21)]. Even more interesting could be that—given that both identified subtypes of estradiol receptors are nuclear receptors—the high-affinity binding essays still show some "blur" in the data, leaving room for a possible third subtype of estradiol receptor. Some investigators have suggested that if such an "ERγ" receptor exists, it could possibly be a membrane-bound protein [e.g., (23)]. Such a receptor could explain the relatively fast behavioral effects of estrogens that have been observed (including the stimulus properties of this steroid). These "rapid" effects of estrogens (within about 3–4 h after systemic administration) are difficult to explain in terms of genomic effects (the same "puzzling" rapid effects also account for testosterone, and to a lesser extent for progesterone).

Another interesting development in the field of estrogens is that a variety of nonsteroidal estrogens have become available [e.g., (19)], showing mixed agonist/antagonist properties. Dependent on the tissue, nonsteroidal estrogens mimic or block the estradiol effects, and this appears to be determined by cellular background and promoter context, but which could also have to do with a differential distribution of ERα and ERβ receptors (or with the involvement of a possible membrane-bound subtype of the estradiol receptor). Virtually nothing is known about CNS effects of these kind of nonsteroidal estrogens, and it would be interesting to investigate discriminative stimulus properties of nonsteroidal estrogens derived from distinct chemical classes (e.g., raloxifene, levormeloxifene, or idoxifene). Characterizing the stimulus properties of these compounds may give interesting insights into their action of the CNS, and might elucidate in more detail the estradiol signaling system.

ACKNOWLEDGEMENTS

The various two-lever DD studies referred to as "unpublished data" were conducted by Thomas Jensen and Hanne Nielsen. The 1996 data were obtained under the supervision of Dr. Michael Swedberg.

REFERENCES

1. Colpaert, F. C.; Koek, W.: Empirical evidence that the state dependence and drug discrimination paradigms can generate different outcomes. Psychopharmacology (Berlin) 120:272–279; 1995.
2. Couse, J. F.; Lindzey, J.; Grandien, K.; Gustafsson, J.-Å.; Korach, K. S.: Tissue distribution and quantitative analysis of estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) messenger ribonucleic acid in the wild-type and ERα-knockout mouse. Endocrinology 138:4613–4621; 1997.
3. De Beun, R.; Heinsbroek, R. P. W.; Slangen, J. L.; Van de Poll, N. E.: Discriminative stimulus properties of estradiol in male and female rats revealed by a taste-aversion procedure. Behav. Pharmacol. 2:439–445; 1991.
4. De Beun, R.; Heinsbroek, R. P. W.; Slangen, J. L.; Van de Poll, N. E.: Dose-dependent and time-related stimulus properties of LHRH in male but not in female rats. Psychoneuroendocrinology 17:71–79; 1992.
5. De Beun, R.; Jansen, E.; Heinsbroek, R. P. W.; Slangen, J. L.; Van de Poll, N. E.: Intracerebroventricular LHRH may serve as a discriminative stimulus in male rats. Psychoneuroendocrinology 17:711–719; 1992.
6. De Beun, R.; Jansen, E.; Slangen, J. L.; Van de Poll, N. E.: Testosterone as appetitive and discriminative stimulus in rats: Sex- and dose-dependent effects. Physiol. Behav. 52:629–634; 1992.
7. De Beun, R.; Peeters, B. W. M.; Broekkamp, C. L. E.: Stimulus characterization of estradiol applying a crossfamiliarization taste aversion procedure in female mice. Physiol. Behav. 53:715–719; 1993.
8. De Beun, R.; Rijk, H. W.; Broekkamp, C. L. E.: Cross-familiarisation conditioned taste aversion procedure as a method to reveal stimulus resemblance between drugs: Studies on the 5-HT_{1A} agonist 8-OHDPAT. Psychopharmacology (Berlin) 112:121–128; 1993.
9. Ebner, D. L.; Richardson, R.; Riccio, D. C.: Ovarian hormones and retention of learned fear in rats. Behav. Neural Biol. 33:45–58; 1981.
10. Gorski, R. A.: Sexual differentiation of the endocrine brain and its control. In: Motta, M., ed. Brian endocrinology. New York: Raven Press Ltd.; 1991:71–104.
11. Gorzalka, B. B.; Wilkie, D. M.; Hanson, L. A.: Discrimination of ovarian steroids by rats. Physiol. Behav. 58:1003–1011; 1995.
12. Gray, P.: Effect of the estrous cycle on conditioned avoidance in mice Horm. Behav. 8:235–241; 1977.
13. Gustavson, C. R.; Gustavson, J. C.; Young, J. K.; Pumariega, A. J.; Nicolaus, L. K.: Estrogen induced malaise. In: Lakoski, J. M.; Perez-Polo, J. R.; Rassin, D. K., eds. Neurology and neurobiology, vol. 50. Neural control of reproductive function. New York: Alan R. Liss Inc.; 1989:501–523.
14. Heinsbroek, R. P. W.; Van Haaren, F.; Zantvoord, F.; Van de Poll, N. E.: Discriminative stimulus properties of pentobarbital and progesterone in male and female rats. Pharmacol. Biochem. Behav. 28:371–374; 1987.
15. Ikard, W. L.; Bennett, W. C.; Lundin, R. W.; Trost, R. C.: Acquisition and extinction of the conditioned avoidance response: A comparison between male rats and estrus and non-estrus female rats. Psychol. Rec. 22:249–254; 1972.
16. Jacobowitz, D. M.: Multifactorial control of pituitary hormone secretion: The "wheels" of the brain. Synapse 2:186–192; 1988.
17. Järbe, T. U. C.: State-dependent learning and drug discriminative control of behaviour: An overview. Acta Neurol. Scand. 74:37–59; 1986.
18. Miele, J.; Rosellini, R. A.: Svare, B.: Estradiol benzoate can function as an unconditioned stimulus in a conditioned taste aversion paradigm. Horm. Behav. 22:116–130; 1988.
19. Mitlak, B. H.; Cohen, F. J.: In search of optimal long-term female hormone replacement: The potential of selective estrogen receptor modulators. Horm. Res. 48:155–163; 1997.
20. Morilak, D. A.; Orndoff, R. K.: Persistence of flavor neophobia as an indicator of state-dependent retention induced by pentobarbital, stress, and estrus. Behav. Neural Biol. 38:47–60; 1983.

21. Osterlund, M.; Kuiper, G. G. J. M.; Gustafsson, J. A.; Hurd, Y. L.: Differential distribution of estrogen receptor-α and receptor-β messenger RNA within the female rat-brain. Mol. Brain Res. 54:175–180; 1998.
22. Overton, D. A.: Historical context of state dependent learning and discriminative drug effects. Behav. Pharmacol. 2:253–264; 1991.
23. Pappas, T. C.; Gametchu, B.; Yannariello-Brown, J.; Collins, T. J.; Watson, C. S.: Membrane estrogen receptors in GH3/B6 cells are associated with rapid estrogen-induced release of prolactin. Endocrine 2:813–822; 1994.
24. Rice, A. G.; Lopez, A.; Garcia, J.: Estrogen produces conditioned taste aversions in rats which are blocked by antihistamine. Soc. Neurosci. Abstr. 13:556; 1987.
25. Roca, C. A.; Schmidt, P. J.; Rubinow, D. R.: Drug discrimination of gonadal steroids in menopausal women. Biol. Psychiatry 41:118S; 1997.
26. Stewart, J.; Krebs, W. H.; Kaczender, E.: State-dependent learning produced with steroids. Nature 216:1223–1224; 1967.
27. Stolerman, I. P.: The drug discrimination bibliography. Internet version: www.iop.bpmf.ac.uk/home/DDD/; 1998.
28. Van Hest, A.; Slangen, J. L.; Olivier, B.: Is the conditioned taste aversion procedure a useful tool in drug discrimination research? In: Oliver, B.; Mos, J.; Slangen, J. L., eds. Animal models in psychopharmacology. Basel: Birkhäuser; 1991:399–406.