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# Effect of Subcutaneous vs Intraperitoneal Administration of an Anti-Estrogen, CI-628, on Estradiol- and Estradiol Benzoate-Stimulated Lordosis in the Ovariectomized Rat[1]

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LANDAU, I. T. *Effect of subcutaneous vs intraperitoneal administration of an anti-estrogen, CI-628, on estradiol- and estradiol benzoate-stimulated lordosis in the ovariectomized rat.* PHARMAC. BIOCHEM. BEHAV. 5(4) 473–476, 1976. – The anti-estrogen CI-628 (3 mg) inhibited estradiol (E, 150 μg, SC in oil)-stimulated lordosis in ovariectomized rats, when the anti-estrogen was given intraperitoneally (IP) at the time of E injection (Hr 0). No lordosis inhibition occurred after similar treatment, if the CI-628 was given subcutaneously. For estradiol benzoate (EB, 2 μg, SC in oil)-stimulated lordosis, CI-628 (Hr 0) had a substantial inhibitory effect whether it was given IP or SC, the greater percentage inhibition occurring after SC CI-628 (Experiment 1). All animals received sequential injections of estrogen and progesterone (500 =s,g, SC in oil) and were then tested to 10 mounts by an intact male. These results suggested that, after E injection, neural tissues mediating lordosis have a shorter period of sensitivity to the behavioral effects of CI-628 than after EB injection. Thus, inhibition of E-stimulated lordosis apparently required the more rapid onset of intracellular estrogen retention inhibition resulting from CI-628 given IP rather than SC. This was not the case for EB-stimulated lordosis, where a longer period of sensitivity to CI-628 seemed likely. This hypothesis was supported by the data of Experiment 2, in which CI-628 (3 mg, IP) was first given 3 hr after E or EB treatment. In this paradigm, CI-628 no longer inhibited E-stimulated lordosis. In contrast, the anti-estrogen's effect on EB-stimulated lordosis was at least equal to the inhibition occurring after CI-628 (IP) given at Hr 0.

Anti-estrogen CI-628 Estradiol Estradiol benzoate Lordosis Sexual behavior

THE lordosis response in ovariectomized rats, elicited after estrogen and progesterone administration, can be inhibited to varying degrees by a class of pharmacologic compounds known as anti-estrogens [2, 12, 14, 15]. In particular, the anti-estrogens CI-628 and MER-25 have been reported to inhibit the facilitatory effects of subcutaneous (SC) injections of estradiol-17β-benzoate (EB) on lordosis [2, 8, 9, 12]. However, two previous reports [9,12] have cited the inability of these two anti-estrogens, when given SC, to inhibit lordosis after a concurrent SC injection of unesterfied estradiol-17β(E).

For neural tissues that mediate lordosis [3,11], data on the biochemical effects of E, EB, and CI-628 suggest a possible explanation for this difference in the behavioral effects of CI-628 after E vs EB injections. Retention of radioactivity in the nuclei of the medial basal hypothalamic-preoptic area (HPOA), after an SC injection of tritiated EB ($^3$H-EB) in ovariectomized rats, persists at near maximal levels for more than 24 hr [8]. This has been correlated with a comparably long period of at least partial sensitivity to the behavioral effects of CI-628 injections [8]. A much shorter total duration of estrogen retention is reported after intraperitoneal (IP) injections of $^3$H-E, with nuclear levels of radioactivity reaching a peak within the first 2 hr and diminishing rapidly thereafter [10]. A similarly brief cell radioactivity retention time also seems likely after SC injected $^3$H-E [6]. Thus, the more pulsatile accumulation and decline in neural tissue estrogen levels after an injection of $^3$H-E (as compared to $^3$H-EB) suggests that the time period during which anti-estrogens may influence the eventual behavioral response may be considerably briefer after E than after EB injection.

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CI-628,when given SC 20 min before an SC injection of $^3$H-EB, did not begin to inhibit HPOA nuclear estrogen retention until more than 3 hr after injection [8]. However, when the same dose of CI-628 was given IP, a more rapid onset of nuclear inhibition of estrogen retention resulted [8]. In both cases, the inhibition persisted for many hours after its initial onset. These results suggest that IP injected CI-628, by nature of its more rapid latency to nuclear estrogen retention inhibition, might be able to inhibit E-stimulated lordosis at a time when SC injected CI-628 would not (e.g. [12]). Evidence of a rapid onset of hypothalamic nuclear estrogen retention inhibition after $^3$H-E by intraperitoneal [15] or intravenous [4] CI-628 injections agrees with this hypothesis. For EB-stimulated lordosis, where the period of sensitivity to anti-estrogens is expected to be longer, both SC and IP injections of CI-628 are likely to be effective (e.g. [2,12]). The present experiments test these predictions.

Note that the doses of E and EB used in this study are very different. This reflects the relatively large difference in threshold doses of the two estrogens required to stimulate lordosis (cf. [12]). The possibility that the relatively large dose of E used may be affecting the ability of CI-628 to inhibit E-stimulated lordosis will be discussed.

## METHODS

### *Experiment 1*

The animals used in this experiment were 31 female Sprague-Dawley rats (Charles River). Animals were housed individually in wire rack cages, supplied with food and water ad lib, and maintained on an LD 14:10 cycle (lights on at 1300). Animals were 250–300 g at the time of bilateral ovariectomy (Equithesin anesthetic; 0.25 cc/100 g body weight). Behavior testing began 2 weeks after ovariectomy, with the animals divided into 4 groups as follows: (1) CI-628(SC)/EB (n = 8); (2) CI-628(IP)/EB (n = 8); (3) CI-628(SC)/EB (n = 8); (4) CI-628(IP)/E (n = 7). The two EB injected groups received –EB (2 $\mu$g, SC in oil) at a time designated as Hr 0, progesterone (500 $\mu$g, SC in oil) at Hr $^+$42, and were tested for lordosis responses to 10 mounts (with thrusting) by an intact male at Hrs $^+$47 to $^+$49. The 2 E injected groups (150 $\mu$g, SC in oil) were similarly treated. The 150 $\mu$g dose of E was chosen after preliminary testing established that this was approximately the minimal dose necessary to bring 100% of control animals into heat (2 $\mu$g of EB similarly brought 100% of control animals into heat). In addition to the E (or EB) and progesterone given on every test, the animal was given 3 mg of CI-628, either SC or IP, on one test, and the 10% ethanol vehicle (SC or IP) on another test. These injections were given 20 min before E or EB (i.e. Hr 0). The 3 mg dose of CI-628 was chosen to equate the CI-628(EB ratio with that used in a previous experiment measuring nuclear radioacitivty retention after $^3$H-EB [8].

Thus, each female was tested twice (with and without CI-628), with the tests separated by 1–2 weeks and the order of testing counterbalanced for experimental treatment. Testing was begun 1 hr after the start of the dark cycle in a 20 in. diameter Plexiglas arena. The arena was illuminated by a 25 W red light. Each male was given at least 5 min in the arena prior to the introduction of the female.

A lordosis quotient = (number of lordoses/number of mounts) x 100 and a measure of mean lordosis intensity (on a scale of 0–3) were determined for each test (see [12]). Differences between separate tests of the same group were evaluated by the Wilcoxon T-test; differences between groups were evaluated by the Mann-Whitney U test.

### *Experiment 2*

The 31 animals from the previous experiment were used again in this study. Each animal received the same hormone, E or EB, as it had been given previously, with group assignment counterbalanced for previous anti-estrogen treatment. Four groups were formed as follows: (1) Vehicle (Hr +3)/EB (n = 8); (2) CI-628(Hr +3)/EB (n = 8); (3) Vehicle (Hr +3)/E (n = 7); (4) CI-628(Hr +3)/E (n = 8). All CI-628 injections (3 mg) were given IP at 3 hr after E or EB injection. Each animal was tested only once. All other details of the testing procedure remain as described above.

## RESULTS

### *Experiment 1*

For E injected animals, CI-628 (3 mg) given SC at Hr 0 was without inhibitory effect (Table 1), in agreement with previous results [12]. However, the lordosis intensity scores of the IP injected CI-628 group receiving E were significantly lower than both its own vehicle injected control scores and those of the CI-628(SC)/E group. The reduced lordosis quotients (LQs) of the CI-628(IP)/E group similarly approached statistical significance (Table 1).

For EB injected animals, LQs and lordosis intensity scores were significantly reduced for both SC and IP CI-628 groups. In contrast to the E-injected animals, the scores of the CI-628(SC)/EB group were significantly lower than those of the CI-628(IP)/EB group (Table 1).

### *Experiment 2*

Even though CI-628 given IP at Hr 0 could inhibit both EB- and E-stimulated lordosis, a shift in the time of anti-estrogen injection was predicted to affect E injected animals more than those receiving EB. This is because the shortened duration of nuclear radioactivity retention after $^3$H-E vs $^3$H-EB [8,10] suggests a shorter period of sensitivity to the behavioral effects of CI-628 after E than after EB injection. This experiment assessed the effects of IP injected CI-628 given 3 hr after either E or EB.

For E injected animals, CI-628 no longer significantly reduced lordosis scores (Talbe 2). Thus, the shift in time of CI-628 (IP) injection to 3 hr after E was enough to prevent CI-628's behavioral inhibitory effects. This is consistent with the need for a rapid neural effect of CI-628, when it is given at Hr 0, if it is to inhibit lordosis (see above).

In contrast, EB injected animals receiving CI-628 (IP) at Hr $^+$3 had mean LQs and lordosis intensity scores below that of any other group in either experiment. While comparisons with the Hr 0 groups of the previous experiment must be made with caution (due to the differences in testing experience and prior hormonal treatment), there is a strong suggestion that the Hr $^+$3 (IP) group receiving EB was inhibited to a greater extent than the CI-628(IP)/EB (Hr 0) group shown in Table 1. Thus, the Hr $^+$3 (IP) CI-628 injections were at least equally effective as Hr 0 injections for the inhibition of EB-stimulated lordosis, perhaps more so.

## DISCUSSION

A 3 mg dose of CI-628, when given IP at Hr 0,

TABLE 1

THE EFFECT OF INTRAPERITONEAL (IP) VS. SUBCUTANEOUS (SC) INJECTED CI-628 (3 mg), GIVEN AT Hr 0, ON E- AND EB-STIMULATED LORDOSIS IN OVARIECTOMIZED RATS

| Group | Hormone | Mean (± SEM) LQ | Mean (± SEM) LI | % of control LQ | % of control LI |
|---|---|---|---|---|---|
| 1. Vehicle (SC) | EB | 97.50 ± 1.64 | 2.13 ± 0.12 | | |
| CI-628 (SC) | EB | 16.25 ± 7.06† | 0.23 ± 0.09† | 17% | 11% |
| 2. Vehicle (IP) | EB | 98.75 ± 1.25 | 1.94 ± 0.10 | | |
| CI-628 (IP) | EB | 43.75 ± 12.53† | 0.80 ± 0.25† | 44% | 41% |
| 3. Vehicle (SC) | E | 98.75 ± 1.25 | 1.86 ± 0.17 | | |
| CI-628 (SC) | E | 76.25 ± 13.35 | 1.31 ± 0.28 | 77% | 70% |
| 4. Vehicle (IP) | E | 85.71 ± 5.71 | 1.27 ± 0.18 | | |
| CI-628 (IP) | E | 37.14 ± 17.00‡ | 0.46 ± 0.22* | 43% | 36% |

*Vehicle vs. CI-628 significant at $p<0.05$, Wilcoxon T.
†Vehicle vs. CI-628 significant at $p<0.01$, Wilcoxon T.
‡Vehicle vs. CI-628; $p<0.06$, Wilcoxon T.
For EB injected animals, SC CI-628 reduced lordosis scores below that of IP CI-628: significant at $p<0.05$ for lordosis intensity; $p<0.06$ for LQ (Mann-Whitney U).
For E injected animals, IP CI-628 reduced lordosis scores below that of SC CI-628: significant at $p<0.05$ for lordosis intensity; $p<0.06$ for LQ (Mann-Whitney U).
E = estradiol-17$\beta$ (150 $\mu$g, SC); EB = estradiol-17 $\beta$-benzoate (2 $\mu$g, SC); Vehicle = 10% ethanol; LQ = lordosis quotient; LI = lordosis intensity; % of control = lordosis inhibition in CI-628 group expressed as a percentage of the scores of corresponding vehicle injected control group.
All animals also received progesterone (500 $\mu$g, SC) 5–7 hr before behavioral testing.

TABLE 2

THE EFFECT OF INTRAPERITONEALLY INJECTED CI-628 (3 mg) GIVEN 3 HR AFTER E OR EB ON LORDOSIS IN OVARIECTOMIZED RATS

| Group | Hormone | Mean (± SEM) LQ | Mean (± SEM) LI | % of control LQ | % of control LI |
|---|---|---|---|---|---|
| 1. Vehicle | EB | 97.50 ± 2.50 | 1.96 ± 0.16 | | |
| 2. CI-628 | EB | 3.75 ± 2.63* | 0.05 ± 0.03* | 4% | 3% |
| 3. Vehicle | E | 92.50 ± 4.12 | 1.15 ± 0.22 | | |
| 4. CI-628 | E | 83.75 ± 8.85 | 1.64 ± 0.23 | 91% | 143% |

*Vehicle vs. CI-628 (for groups receiving same hormone) significant at $p<0.001$, Mann-Whitney U.
EB = estradiol-17$\beta$-benzoate (2 $\mu$g, SC); E = estradiol-17$\beta$ (150 $\mu$g, SC); Vehicle = 10% ethanol; LQ = lordosis quotient; LI = lordosis intensity; % of control = lordosis inhibition in CI-628 group expressed as a percentage of the scores of corresponding vehicle injected group control.
All animals also received progesterone (500 $\mu$g, SC) 5–7 hr before behavioral testing.

substantially inhibited E-stimulated lordosis scores. Further, in agreement with previous results [12], SC administered CI-628 had no significant effect on E-stimulated lordosis (Table 1). These results, together with the data on nuclear estrogen retention [8, 10, 15] detailed in the Introduction, suggest that the period during which CI-628 must antagonize the effects of E in neural tissues mediating lordosis (e.g. HPOA) is relatively short, if this antagonism is to have a behavioral effect. Thus, the more rapid onset of nuclear estrogen retention inhibiton after IP injected CI-628, as opposed to SC injections, is apparently necessary to significantly affect this brief sensitive period.

In contrast, CI-628, given either IP or SC, significantly reduced lordosis scores of EB injected animals, with SC CI-628 causing the greater percentage inhibition (Table 1). This is consistent with the hypothesis that the long period of nuclear estrogen retention after EB injection [8] implies a longer period of sensitivity to the effects of anti-estrogens than after E injection. Thus, for the inhibition of EB-stimulated lordosis, the rapid onset of IP injected CI-628's (Hr 0) nuclear inhibitory effects is neither essential nor perhaps even desirable; it is the slower onset of nuclear estrogen retention inhibition after SC injected CI-628 and the long persistance of this inhibition in both cases that is most likely of greater importance.

Similarly, in Experiment 2, Hr +3(IP) injections of CI-628 reduced EB-stimulated lordosis responses to a lower level than for any Hr 0 injected group in Experiment 1. This agrees with the proposed importance of biochemical effects beyond the initial 3 hr period after EB injection for the eventual inhibition of EB-stimulated lordosis. In contrast, IP injections of CI-628, when given at Hr+3, were no longer effective in inhibiting E-stimulated lordosis (Table 2). This agrees with the proposed brief period of sensitivity to CI-628 for E-stimulated lordosis. CI-628 given IP 1 hr or more before E injection is apparently equally ineffective in inhibiting lordosis (Landau, unpublished data). This suggests the importance of a concurrent presence of initial

levels of CI-628 and E for the inhibition of lordosis that is not necessary for EB-stimulated lordosis [8].

However, when E is given intravenously (IV) [15,17], CI-628 can inhibit lordosis over a much wider range of injection times. While the exact reason for this inconsistency with the present results is not clear, it may be related to the fact that, in the previous studies, behavior testing began around 24 hr after E injection, rather than 48 hr. Also of possible importance is the much lower behavioral threshold dose of E required when it is given IV.

Powers [12] has proposed that, because estradiol levels return to baseline in plasma [16] and brain [1, 5, 6, 8, 10, 15] faster after E injections than after EB, higher doses of E relative to EB would be necessary to maintain sufficient estradiol levels for the minimum duration required for the stimulation of lordosis (cf. [7,13]). This would result in an initial large excess of estradiol after E injection with which CI-628 could not effectively compete. This would account for both the higher behavioral threshold dose of E relative to EB and the inability of CI-628 (SC; Hr 0) to inhibit E-stimulated lordosis [12].

However, the present results suggest that it is the delayed HPOA nuclear inhibitory effects of CI-628 given SC, rather than specifically the high E dosage, that prevents CI-628's inhibition of lordosis. This is seen as why the same dose of CI-628, when given IP, was able to inhibit E-stimulated lordosis (Experiment 1). The findings that: (1) 3 mg of CI-628, given IP at Hr 0, could only partially antagonize EB-stimulated lordosis yet could also inhibit the behavioral responses to a dose of E that was 75 times higher than the dose of EB used in this study (Table 1) and (2) for E-stimulated lordosis, IP CI-628 caused greater inhibition than SC CI-628, while the reverse was true for EB stimulated lordosis, further suggest that the differences between E and EB responsiveness to CI-628 are not simply a matter of higher E dose used or that CI-628 given IP may result in an initially higher effective dose than when given SC.

In summary, results suggest that the difference in behavioral threshold doses for E vs EB is related to the shorter period of intracellular estrogen retention after E injection [8, 10, 12]. (This is not meant to imply that EB itself is retained intracellularly longer than E. Instead, results [5] suggest that EB is rapidly metabolized to E upon entering systemic circulation, and that the increase in total duration of radioacitvity present is due to the benzoate moiety causing a slower release of the hormone from the SC injection site.) This higher required E dose is likely to make it more difficult for CI-628 to inhibit E-stimulated lordosis as opposed to EB-stimulated lordosis ([12] and Experiment 1). Despite this, CI-628 can still effectively inhibit E-stimulated lordosis if the anti-estrogen is given at a time (Hr 0) and by a route of administration (IP) likely to affect initial nuclear accumulation of estrogens in neural tissues ([8] and Experiment 1). For EB-stimulated lordosis, CI-628 was generally more effective over a wider range of injection times, indicating a longer period of behavioral sensitivity to CI-628 after EB (than after E) injection ([8] and Experiment 2). The results support an importance of the continued presence of estrogen in neural tissue for the eventual initiation of lordosis (i.e. maintenance function) after EB, but not after E, injection. This may be because initially higher intracellular estrogen levels after SC E (vs EB) injections lead to an earlier termination of the period during which estrogen directly acts to mediate an eventual behavioral response.

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