Pharmacology Biochemistry & Behavior, Vol. 16, pp. 93–99, 1982. Printed in the U.S.A.

Modulation of Food Intake by Hypothalamic Implants of Estradiol Benzoate, Estrone, Estriol and CI-628 in Female Rats[1]

THOMAS P. DONOHOE AND ROBIN STEVENS

Department of Psychology, University Park
University of Nottingham, Nottingham, UK NG7 2RD

Received 9 April 1981

DONOHOE, T. P. AND R. STEVENS. *Modulation of food intake by hypothalamic implants of estradiol benzoate, estrone, estriol and CI-628 in female rats.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 93–99, 1982.—Ovariectomised rats were implanted unilaterally with cannulae aimed at the ventromedial nucleus-arcuate region of the hypothalamus. Crystalline implants of estradiol benzoate and of the antiestrogenic compound CI-628 over a 72-hour stimulation period caused significantly greater food intake reductions than did implants of cholesterol. More dorsal and lateral placements were generally ineffective in reducing food intake. Implants of estrone and estriol produced equivalent reductions in food intake and body weight to those produced by estradiol benzoate. The possible molecular mode of action is discussed.

Food intake Estradiol benzoate CI-628 Estrone Estriol Ventromedial hypothalamus

IN order to investigate the neural loci at which estrogen may influence food intake, intracranial implants of crystalline estradiol benzoate [EB] have been used in several studies. Implants into the ventromedial area of the hypothalamus [VMH] reduce food intake and body weight in ovariectomised rats [3, 11, 25] whereas estrogen implants into other hypothalamic areas are much less effective. Systemic injections of the non-steroidal, anti-estrogenic compound MER-25 can block estradiol-induced sexual receptivity [14] and locomotor activity [19] possibly by competing with estradiol for target receptor sites. However, MER-25 reduces food intake in ovariectomised rats when administered systemically or implanted directly into the VMH [21]. This dual action of MER-25 may reflect some particular property of the anti-estrogen molecule or as suggested by Wade [23], the neural estrogen 'receptor' affecting body weight may differ from other estrogen-sensitive systems.

Nitromifene citrate (CI-628) is believed to antagonise peripheral estrogenic action while not interfering with estrogen uptake in central nervous system sites [26]. If estrogen affects food intake by peripheral action, then CI-628 should mimic the effects of ovariectomy on food intake, i.e. increase it, yet King and Cox [13] found to the contrary that systemic injections of CI-628 reduced food intake, suggesting that ovariectomy increases food intake due to diminished activity at some central sites. However, CI-628 could act directly at one or more of the central sites to reduce food intake.

The first experiment compares the anorexic and weight reducing effects of intrahypothalamic implants of crystalline EB and CI-628.

Fishman [8] has suggested that estradiol may only be a prohormone which is converted to the active agent at the site of action. This raises the possibility that some of the systemic metabolites of estrogen may be more effective in reducing food intake in female rats than estradiol itself. The systemic metabolism of estradiol involves a rapid and partially reversible oxidation to estrone [9] which can then be subject to competitive hydroxylations at carbon 16 (leading to estriol) or at carbon 2 (leading to the catecholestrogen, 2-hydroxyestrone). When estrone was administered systemically to ovariectomised rats it was only one tenth as effective as estradiol in reducing food intake [22]. However, different rates of systemic metabolism, rates of blood brain barrier transfer and in situ transformations in the central nervous system (including the interconversion of estrone and estradiol) may make comparisons of the effectiveness of estrogens administered systemically rather complex [8].

In order to overcome these problems of systemic administration, the effects of estradiol and two of its major metabolites (estrone and estriol) on food intake and body weight are also evaluated by utilising intrahypothalamic crystalline implant techniques.

METHOD

Subjects

Sixty-one female, Wistar rats weighing approximately 190 g were received from Charles River U.K. Twenty-five of these animals were tested in the first phase of the experiment (comparing the effects of CI-628 and EB), the remainder were used to compare the actions of estradiol metabolites in

[1]This research was funded by a M.R.C. grant to T. P. Donohoe.

TABLE 1

THE NUMBER OF OVARIECTOMISED RATS SHOWING CHANGES IN FOOD INTAKE DURING INTRAHYPOTHALAMIC STIMULATION WITH ESTRADIOL BENZOATE, CI-628, CHOLESTEROL, ESTRONE AND ESTRIOL

	Number of rats responding with change in food intake.			
	Increase	Decrease		
		<10%	10–20%	>20%
Treatment (N=21)				
Estradiol Benzoate	0	8	6	7
CI-628	0	5	6	10
Cholesterol	3	11	7	0
Treatment (N=23)				
Estradiol Benzoate	0	9	8	6
Estrone	2	6	5	10
Estriol	2	2	6	13

the second phase. The animals were housed in individual cages with Pilsbury rodent chow pellets and tap water available ad lib. A 12:12 hour light-dark cycle (lights on at 0730 hr) was maintained thoughout the experiment. The temperature was thermostatically maintained at approximately 23°C.

Surgery

Three days after arrival, all animals were ovariectomised via bilateral dorsolateral incisions under ether anaesthesia. Twenty-five days later, the animals were anaesthetised with sodium pentobarbital (45 mg/kg) and stereotaxically implanted with unilateral double walled stainless steel cannulae (inner: 31 gauge, outer: 23 gauge). The guide cannulae were secured to the skull with four No. 80 machine screws and acrylic cement (Cranioplast, Roanoke Plastic Products, Virginia, U.S.A.). The cannulae were aimed at the VMH-arcuate region using the following co-ordinates, according to Pellegrino and Cushman [16], anterior 5.4, lateral −0.8 and vertical −3.0. Three animals died following stereotaxic surgery, two of these were in the first phase of the experiment.

Procedure

Phase 1. Five days after stereotaxic surgery and continuing throughout the experiment, food intake and body weight were measured to the nearest 0.5 g at approximately 0900 hr each day. Spillage appeared to be negligible.

Three weeks later, the drug treatments were started, each animal received all treatments but the order of treatments was randomized. The inner cannulae were exchanged for clean 31 gauge stainless steel cannulae which had been tapped into crystalline EB or cholesterol (Sigma, Poole, U.K.) or CI-628 (Warner-Lambert, Michigan, U.S.A.). Prior to insertion, the outside of the cannulae were scraped with a scalpel blade and examined to ensure that only the lumen contained hormone. The exchange of cannulae occurred at approximately 1000 hr and animals were exposed for 72 hours of continuous stimulation from the drug. On the third day of treatment, vaginal smears were taken from each animal. The inner cannulae were replaced by empty ones for the next 10 days following which the above treatments were repeated until all animals had received each drug.

Phase 2. Thirty-five animals took part in this phase of the experiment. The procedures used were similar to those just described except that 11 days of recovery were interspersed between 3 days of hormone treatment. Moreover, the 3 steroids used were estradiol benzoate, estrone and estriol. (Cholesterol was not repeated to avoid the problem of too many implants at the same site.)

Histology

After the animals were killed with an overdose of sodium pentobarbital they were perfused pericardially with 0.9% NaCl solution followed by neutralised 10% Formalin solution and the brains were removed and fixed in Formalin. Coronal sections were cut at 10 μ and every tenth was saved and stained with toluedine blue. The sections were examined microscopically to determine cannula placements, and cannula placements were mapped on to diagrams redrawn from the atlas of Pellegrino and Cushman [16].

RESULTS

Table 1 shows the changes in food intake during intrahypothalamic stimulation with EB, CI-628 and cholesterol. The change in food intake was computed as the percentage change over the 72 hr stimulation period compared to the mean of the 5 day prestimulation intake. Since the vaginal smear of one animal suggested that EB was leaking into systemic circulation and another animal became ill following its third intracerebral injection, the data for only twenty-one animals are presented. The statistical comparisons reported are all based on a within subjects design and the test used was a Wilcoxon matched-pairs test.

Both EB and CI-628 decreased food intake significantly

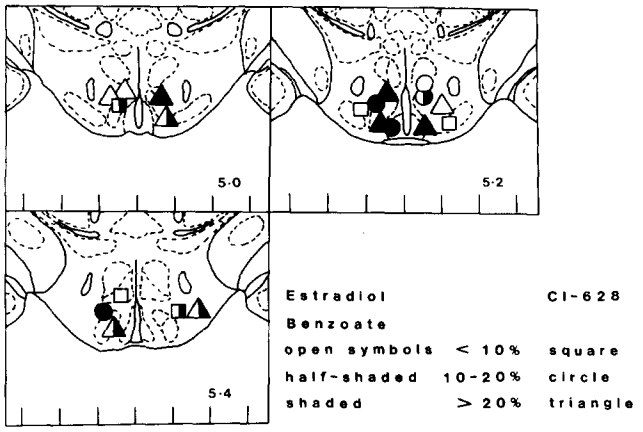

FIG. 1. Coronal sections of the relevant part of the rat brain adapted from Pellegrino and Cushman [16] indicating the location of the EB and CI-628 placements and their effectiveness in decreasing food intake.

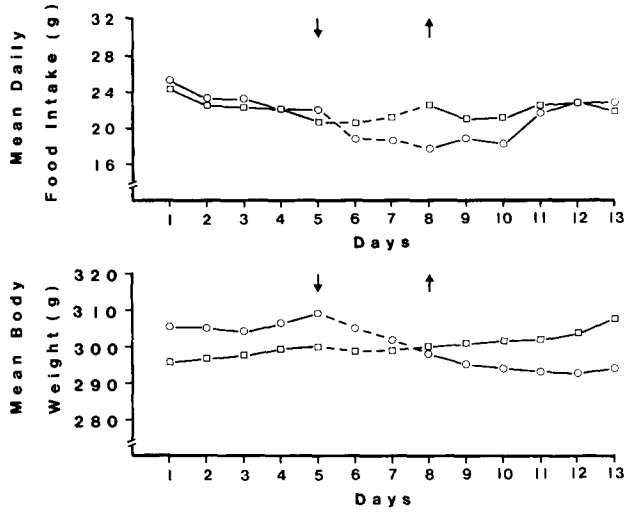

FIG. 2. Mean food intake and body weight of the ovariectomised rats before (Days 1–5), during (Days 6–8) and after (Days 9–13) intrahypothalamic stimulation with estradiol benzoate (clear circles) at effective placement sites (N=13) and cholesterol (clear squares) in the same animals. Descending arrow—start of treatment. Ascending arrow—termination of treatment.

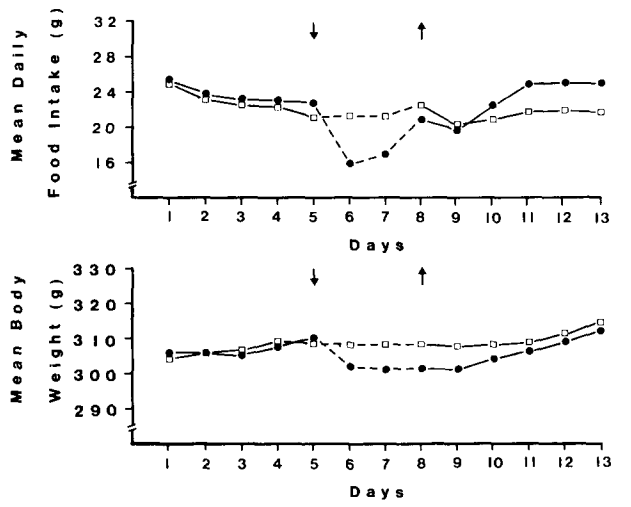

FIG. 3. Mean food intake and body weight of the ovariectomised rats before (Days 1–5), during (Days 6–8) and after (Days 9–13) intrahypothalamic stimulation with CI-628 (filled circles) at effective placement sites (N=16) and cholesterol (clear squares) in the same animals. Descending arrow—start of treatment. Ascending arrow—termination of treatment.

more than cholesterol ($p<0.01$) however all substances significantly reduced food intake during the time it was in the brain compared to prestimulation intakes ($p<0.01$ in each comparison). Figure 1 shows coronal sections of the relevant part of the rat brain adapted from Pellegrino and Cushman [16] indicating the location of the EB and CI-628 placements and their effectiveness in decreasing food intake. A placement was considered 'effective' if the EB or CI-628 produced a reduction in food intake of >10% compared to prestimulation intakes.

The most effective implants were in the VMH-arcuate region while more lateral and dorsal placements were generally ineffective for both EB and CI-628. However, one more lateral placement was an effective site and because of tissue fragmentation, it was impossible to locate the placement of one other animal.

Figure 2 shows the mean food intake and body weight of the ovariectomised rats before (Days 1–5), during (Days 6–8) and after (Days 9–13) intrahypothalamic stimulation with EB and cholesterol.

In the effective EB placements (13 animals), food intake was lower during the first day of exposure to EB than on the preceding 5 day mean for 11 of the animals and for all of them by the second day. On the third day of stimulation food intake continued to decrease for 7 animals, stabilised for 2 animals and increased for 4 animals. When the cannulae containing EB were removed, food intake increased and stabilised at a level near that of the prestimulation period.

In the effective CI-628 placements (16 animals), food intake was lower during the first day of exposure to CI-628 than on the preceding 5 day mean for all animals. On the second day of stimulation, food intake continued to decrease for 6 animals, stabilised for 1 animal and increased for 7 animals, whilst on the third day, food intake decreased for 1 animal, stabilised for another and increased in 14 animals. Again, when the cannulae containing CI-628 were removed, food intake increased and stabilised at a level near that of the prestimulation period.

There was no significant difference between EB and CI-628 in reducing food intake. In 11 animals, both treatments produced >10% reduction in food intake. In these animals, CI-628 produced a larger mean reduction than EB on the first day of stimulation compared to the preceding 5 day mean (−30.2% compared to −21.4%) however the difference was not significant.

Changes in body weight were calculated as the difference

between the mean body weights over the 72 hour stimulation period and compared to the body weights on the day preceding stimulation. In the effective EB placements, body weight was significantly reduced over the 72 hour stimulation period ($p<0.01$). On the first day of exposure to EB, body weight declined in 8 animals, increased in 2 animals and remained unchanged in 3 animals. On the second day, body weight declined further in 9 animals, increased in 3 animals and remained unchanged in 1 animal. On the third day of stimulation, body weight declined for 10 animals, increased for 2 animals and stabilised for another. When the cannulae containing EB were removed, body weights continued to decline in several animals and by Day 13 only 1 animal had returned to its prestimulation body weight. However, subsequent treatments were not initiated for another 6 days to allow the animals to recover the weight loss. There was no correlation between body weight and percentage reduction in food intake during EB treatment.

In the effective CI-628 placements, body weight was also significantly reduced over the 72 hour stimulation period ($p<0.01$). On the first day of exposure to CI-628, body weight declined in 12 animals, and increased in 4 animals, on the second and third day of stimulation, body weight declined in 8 animals and increased in 8 animals. When the cannulae containing CI-628 were removed, body weights stabilised and then increased. Thirteen animals had reached prestimulation body weight by Day 13, but subsequent treatments were delayed for another 6 days. There was no correlation between body weight and percentage reduction in food intake during CI-628 treatment. There was also no significant difference between EB and CI-628 in reducing body weight. Animals receiving cholesterol did not show a significant change in body weight.

Vaginal smears taken from each animal on the third day of stimulation were anestrous in all except one animal (whose data has been omitted from analysis) suggesting that little if any EB was leaking into the systemic circulation.

Table 1 also shows the change in food intake during intrahypothalamic stimulation with EB, estrone and estriol. Due to cannulae becoming blocked, positive vaginal smears or illness, several animals had to be omitted hence the data for only 23 animals are presented.

Each treatment significantly reduced food intake during intrahypothalamic stimulation compared to prestimulation intake ($p<0.01$ in each comparison) but there was no significant difference between EB, estrone or estriol in their ability to reduce food intake when the percentage reductions were compared across treatments. Figure 4 shows coronal sections of the relevant part of the rat brain adapted from Pellegrino and Cushman [16] indicating the location of the estrone and estriol placements and their effectiveness in decreasing food intake.

A placement was again considered effective if the hormone produced a reduction in food intake of >10% compared to prestimulation intake.

The most effective placements were in the ventromedial arcuate region of the hypothalamus although one effective placement was more lateral to this area. Because of fragmentation of tissue, placement sites are shown for only 19 animals.

Figure 5 shows the mean food intake and body weight of the ovariectomised rats before (Days 1–5), during (Days 6–8) and after (Days 9–13), intrahypothalamic stimulation with each treatment. Food intake was lower during the first day of exposure to EB than on the preceding 5 day mean for 22 of the 23 animals. On the second day of stimulation, food intake further declined for 8 animals, stabilised for 3 animals and increased for 12 animals, while on the third day of stimulation, food intake declined for 13 animals, stabilised for 2 animals and increased for 8 animals.

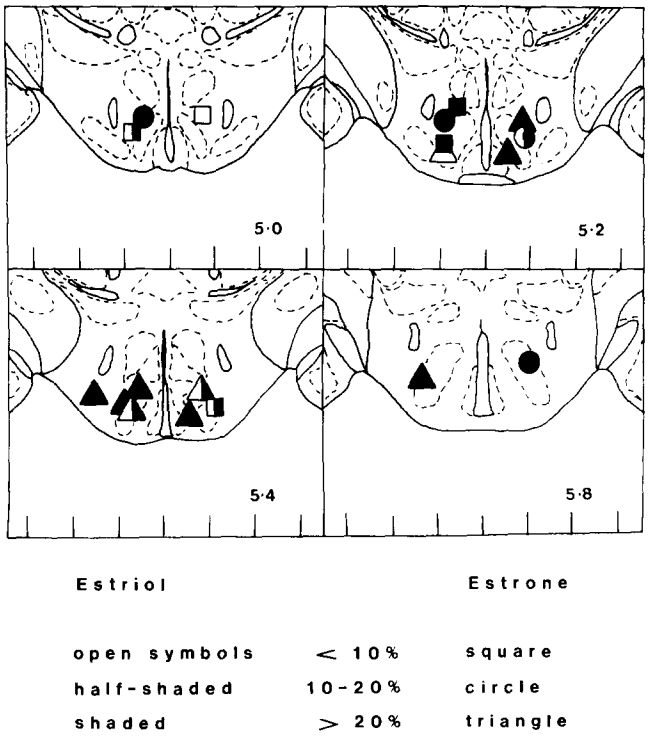

FIG. 4. Coronal sections of the relevant part of the rat brain adapted from Pellegrino and Cushman [16] indicating the location of the estrone and estriol placements and their effectiveness in decreasing food intake.

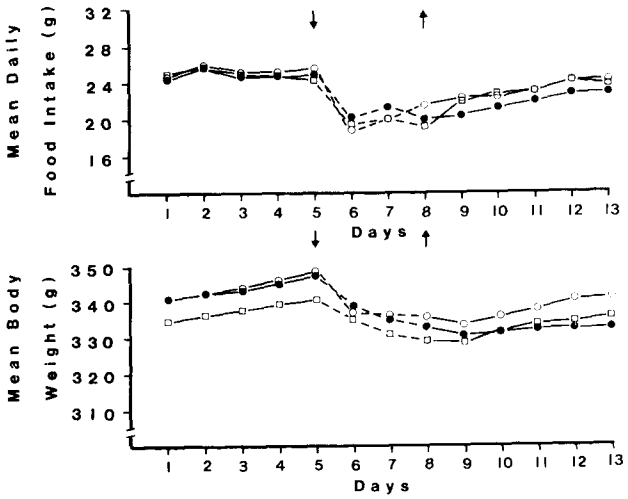

FIG. 5. Mean food intake and body weight of the ovariectomised rats before (Days 1–5), during (Days 6–8) and after (Days 9–12) intrahypothalamic stimulation with estradiol benzoate (filled circles), estrone (clear circles) and estriol (clear squares). Descending arrow—start of treatment. Ascending arrow—termination of treatment.

On the first day of exposure to estrone, food intake was lower than on the preceding 5 day mean for 20 animals, on the second day of stimulation, food intake declined for 7 animals, stabilised for 3 animals and increased for 13 animals. On the third day of stimulation, food intake decreased for 8 animals, stabilised for 2 animals and increased for 13 animals.

A similar pattern was observed for estriol implants, in that food intake was lower on the first day of exposure than on the preceding 5 day mean for 22 of the 23 animals, while on the second day of stimulation, food intake decreased for 11 animals, stabilised for 2 animals and increased for 10 animals. On the third day of estriol implants, food intake decreased for 12 animals, stabilised for another and increased for 10 animals. Each treatment significantly reduced food intake compared to the preceding 5 day mean on the first day of treatment ($p<0.01$). However, there was no significant difference between the treatments. When the cannulae containing the hormone were removed, food intake increased and stabilised at a level similar to that of the prestimulation period.

Body weight was significantly reduced over the 72 hour stimulation period by each treatment ($p<0.01$) compared to the body weights on the day preceding stimulation. There was no significant difference between treatments in their ability to reduce body weight. On the first day of exposure to EB, body weight declined for 20 animals, remained unchanged for another and increased for 2 animals, while on the second day, body weight continued to decline for 17 animals, stabilised for 1 animal and increased for 5 animals. On the third day of stimulation, body weight declined for 13 animals, stabilised for 4 animals and increased for 6 animals. When the cannulae containing EB were removed, body weight initially declined but then increased before stabilising in several animals. By Day 13, only 3 animals had returned to their prestimulation body weights. However, subsequent treatments were not initiated for another 6 days, to allow further time for recovery.

On the first day of exposure to estrone, body weight declined for 18 animals, remained unchanged for 3 animals and increased for 2 animals compared to prestimulation body weights, while on the second day of stimulation, body weight declined for 9 animals, stabilised for 5 animals and increased for 9 animals. On the third day of stimulation, body weight continued to decline for 15 animals, stabilised for 2 animals and increased for 6 animals. When the cannulae containing estrone were removed, body weights stabilised and then increased. By Day 13, 10 animals had returned to their prestimulation body weights.

Body weight declined for 19 animals on the first day of exposure to estriol, remained unchanged for another and increased for 3 animals compared to prestimulation body weights, while on the second day of stimulation body weight declined for 18 animals and increased for 5 animals. On the third day of stimulation, body weight continued to decline for 14 animals and increased for 9 animals. When the cannulae containing estriol were removed, body weights stabilised and then increased. By Day 13, 8 animals had returned to their prestimulation body weights. There was no correlation between body weight and percentage reduction in food intake during EB, estrone and estriol treatments.

DISCUSSION

These results confirm the findings of other studies that estrogens may act directly on neurons in the VMH to decrease food intake. The reduction in food intake with cholesterol implants were small by comparison, but are still surprising since other studies [11,25] found little or no effect with cholesterol. Jankowiak and Stern [11] for instance found cholesterol reduced food intake by >10% in only 2 out of 10 animals. These reductions from cholesterol implants are likely to be due to the implant procedure itself rather than the action of the substance.

Food intake was decreased by intrahypothalamic EB within 24 hours, although the effect may be even more rapid [25]. The most effective placements for EB were in the VMH-arcuate region. Apart from one more lateral placement, dorsal and lateral placements were generally ineffective in reducing food intake. Although the effects of EB implants may have been due to leakage into the ventricles and thus to other neural sites, in all except one animal the histology and anestrous vaginal smears suggest this to be unlikely.

The 'antiestrogen' CI-628 also reduced food intake when implanted into the VMH-arcuate region. Effective placements for CI-628 were generally the same as for EB. (Exceptions may be due to debris in the guide cannulae preventing diffusion of the substance into neural tissue which is a potential problem in the comparison of multiple intracranial injections at the same site).

Wade [22] showed that progesterone treatment attenuated the effects of EB on food intake and body weight in ovariectomised rats in a dose dependent fashion and suggested that high progesterone titers may functionally ovariectomise rats by blocking the effects of estradiol. There is some suggestion that high progesterone levels can interfere with the uptake and binding of estradiol by brain cells [1]. If CI-628 was having its estrogenic effect on feeding behaviour by occupying the neural estrogen "receptor" affecting body weight then we might predict that high levels of progesterone might attenuate its effect on food intake. Donohoe and Stevens (unpublished observations) found that concurrent progesterone treatment did not attenuate the effects of central CI-628 implants on food intake when it was administered systemically (2.5 mg/day SC in 0.15 ml oil).

Unlike systemic administration, estrone was found to be equally effective as EB in reducing food intake when implanted directly into the hypothalamus. This could be because both can be converted to 2-hydroxyestrone in the hypothalamus [10], a metabolite which has been shown to reduce food intake when administered systemically (Garrattini and Fishman; unpublished observations, cited in [8]). However the product of the alternate hydroxylation, estriol, was also found to be effective in decreasing feeding during intrahypothalamic administration.

Although all of the agents tested reduced food intake and/or body weight during the 3 day period of treatment, there were considerable differences in duration of effectiveness, however, little is known about how steroids dissociate from their receptors in vivo or how steroid hormone action is terminated.

The most effective placements were in the ventromedial-arcuate region of the hypothalamus, although one effective placement was more lateral. In some animals, an effective placement for one treatment was not always effective for a later treatment which suggests that debris/gliosis was preventing diffusion of the substance into neural tissue. However, since a random order of treatments was used, it would seem fair to assume that each substance was potentially equally effective in reducing food intake. Wade [22] found that whereas the transient hypophagia and weight loss fol-

lowing systemic injections of estrone and estradiol were similar, the effect of the principal metabolite of estradiol was less effective in influencing behaviour than its precursor. He suggested [23] that it was unlikely that either estradiol or progesterone must be converted to an active metabolite in the target tissues and concluded that "perhaps the significance of the estradiol and progesterone metabolism is simply that the metabolites are not active forms and have little effect on behavior." The present experiment would suggest that this may not be true for estradiol.

The studies of Etgen ([6,7], personal communication) suggest that it is the cell nucleus which is the critical locus of receptor mediated interaction which underlie estrogenic and antiestrogenic effects. Her work suggests that estrogens and antiestrogens promote different conformational or allosteric changes on binding to the receptor protein, and also that the neural chromatin has multiple estrogen acceptor sites, each of which could mediate estrogenic regulation of different neuroendocrine and behavioural functions. Thus synthetic antiestrogens such as MER-25 and nafoxidine have been shown to antagonise estrogen-induced lordosis behaviour but mimic estrogenic regulation of feeding behaviour and body weight [19,20] and to both mimic and antagonise estrogenic regulation of gonadotrophin secretion [2, 4, 5, 18].

This model of estrogenic action further implies that the estrogen receptor acts like an allosteric gene regulator in that the conformational state adopted by the receptor protein when it binds a ligand, determines its functional activity. Although antiestrogens such as CI-628 probably promote receptor conformations which decrease estradiol binding, it also appears that the CI-628-receptor complex can bind to some estrogen-receptor sites since for instance CI-628 implants into VMH reduced feeding. Occupation of the estrogen site by CI-628 could produce allosteric modifications in the receptor which alters the structure or availability of the estrogen binding site.

Although a possible explanation for the finding that estradiol, estrone and estriol have equivalent effects in reducing food intake and body weight is that each substance can bind to appropriate neural chromatin acceptor sites in the hypothalamus, we cannot rule out the possibility that one or more metabolites of these substances are the effective agents. The answer to this question must await further neurochemical investigations. However, this experiment provides further support for a central mechanism through which estrogen may modulate food intake. Whether the peripheral metabolic effects of estrogen [24] are primary or secondary to the central effects can not be determined yet. A recent study by Nunez *et al.* [15] showed that estradiol implants in the VMH caused reductions in food intake without affecting lipoprotein lipase activity or cytoplasmic progestin receptor levels suggesting that the central action might be sufficient to reduce food intake in ovariectomised rats. It sould be noted that hypothalamic implants of the type used by Nunez *et al.* [15] and in the present study do not represent physiological conditions. Ramirez [17] has reported that under physiological conditions EB-induced changes in lipoprotein lipase activity appear before the changes in food intake.

The effects observed could have been due to leakage into the ventricles and thus to neural sites remote from those of original stimulation. The histology and anestrous vaginal smears suggest this was unlikely. However, in the absence of direct measurements of blood estrogen levels and radioactive tracing, it is impossible to exclude that the hormones diffused away from the original placement site.

ACKNOWLEDGEMENT

We wish to acknowledge the excellent technical assistance of Lynne Easom and Teresa Mather, Lesley Rollitt for histological preparations and diagrams, Sam Grainger for photography, Dennis Vardy for cannulae constructions, and Maggie Broom for typing the manuscripts.

REFERENCES

1. Anderson, C. H. and G. S. Greenwald. Autographic analysis of estradiol uptake in the brain and pituitary of the female rat. *Endocrinology* **85:** 1160–1165, 1969.
2. Bainbridge, J. G. and A. P. Labhsetwar. The role of estrogens in spontaneous ovulation: Location of site of action of positive feedback of estrogen by intracranial implantation of the anti estrogen, ICI 46474. *J. Endocr.* **50:** 321–327, 1971.
3. Beatty, W. W., D. A. O'Briant and T. R. Vilberg. Suppression of feeding by intrahypothalamic implants of estradiol in male and female rats. *Bull. Psychon. Soc.* **3:** 273–274, 1974.
4. Callantine, M. R., R. R. Humphrey, S. L. Lee, B. L. Windsor, N. H. Schottin and O. P. O'Brien. Action of an estrogen antagonist on reproductive mechanisms in rat. *Endocrinology* **79:** 153–167, 1967.
5. Dannies, P. S., P. M. Yen and A. H. Tashjian, Jr. Antiestrogenic compounds increase prolactin and growth synthesis in clonal strains of rat pituitary cells. *Endocrinology* **101:** 1151–1156, 1977.
6. Etgen, A. M. Antiestrogens: Effects of tamoxifen, nafoxidine and CI-628 on sexual behaviour, cytoplasmic receptors and nuclear binding of estrogen. *Hormones Behav.* **13:** 97–112, 1979.
7. Etgen, A. M. PhD. Thesis. University of California, 1980.
8. Fishman, J. Appetite and sex hormones. In: *Appetite and Food Intake, Life Sciences Research Report No. 2,* edited by T. Silverstone. Berlin: Dahlem Konferenzen, 1976, pp. 207–218.
9. Fishman, J., H. L. Bradlow and T. F. Gallagher. Oxidative metabolism of estradiol. *J. biol. Chem.* **235:** 3104–3107, 1960.
10. Fishman, J. and B. Norton. Catecholestrogen formation in the central nervous system of the rat. *Endocrinology* **96:** 1054–1057, 1975.
11. Jankowiak, R. and J. J. Stern. Food intake and body weight modifications following medial hypothalamic hormone implants in female rats. *Physiol. Behav.* **12:** 875–879, 1974.
12. Kazuma, H. and C. Loncope. *In vivo* studies on the metabolism of estrone and estradiol by the brain. *Steroids* **23:** 469–481, 1974.
13. King, J. M. and V. C. Cox. Effects of ergocornine and CI-628 on food intake and taste preferences. *Physiol. Behav.* **16:** 111–113, 1976.
14. Meyerson, B. J. and L. Lindstrom. Effects of an estrogen antagonist ethamoxytriphetol (MER-25) on estrous behaviour in rats. *Acta Endocr.* **59:** 41–48, 1968.
15. Nunez, A. A., J. M. Gray and G. N. Wade. Food intake and adipose tissue lipoprotein lipase activity after hypothalamic estradiol benzoate implants in rats. *Physiol. Behav.* **25:** 595–598, 1980.
16. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton-Century-Crofts, 1967.
17. Ramirez, I. Estradiol-induced changes in lipoprotein lipase, eating, and body weight in rats. *Am. J. Physiol.* **240:** E533–E538, 1981.
18. Roy, S., V. B. Mahesh and R. B. Greenblatt. Effects of clomiphene on the physiology of reproduction in the rat. III. Inhibition of uptake of radioactive estradiol by the uterus and pituitary gland of immature rat. *Acta Endocr.* **47:** 669–675, 1964.

19. Roy, E. J. and G. N. Wade. Estrogenic effects of an antiestrogen, MER-25 on eating and body weight in rats. *J. comp. physiol. Psychol.* **90:** 156–166, 1976.
20. Roy, E. J. and G. N. Wade. Role of food intake in estradiol-induced body weight changes in female rats. *Hormones Behav.* **8:** 265–274, 1977.
21. Roy, E. J., C. A. Maass and G. N. Wade. Central action and a species comparison of the estrogenic effects of an antiestrogen on eating and body weight. *Physiol. Behav.* **18:** 137–140, 1977.
22. Wade, G. N. Some effects of ovarian hormones on food intake and body weight in female rats. *J. comp. physiol. Psychol.* **88:** 183–193, 1975.
23. Wade, G. N. Sex hormones, regulatory behaviors and body weight. *Adv. Stud. Behav.* **6:** 201–279, 1976.
24. Wade, G. N. and J. M. Gray. Gonadal effects on food intake and adiposity: a metabolic hypothesis. *Physiol. Behav.* **22:** 583–593, 1979.
25. Wade, G. N. and I. Zucker. Modulation of food intake and locomotor activity in female rats by diencephalic hormone implants. *J. comp. physiol. Psychol.* **72:** 328–336, 1970.
26. Whalen, R. E. and B. B. Gorzalka. Effects of an estrogen antagonist on behaviour and on estrogen retention in neural and peripheral target tissues. *Physiol. Behav.* **10:** 35–40, 1973.