Pharmacology Biochemistry & Behavior, Vol. 31, pp. 425–429. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

The Effects of the Kappa Agonist PD-117302 on Feeding Behaviour in Obese and Lean Zucker Rats

G. E. LEIGHTON, R. G. HILL AND J. HUGHES

Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge, CB2 2QB

Received 25 April 1988

LEIGHTON, G. E., R. G. HILL AND J. HUGHES. *The effects of the kappa agonist PD-117302 on feeding behaviour in obese and lean Zucker rats.* PHARMACOL BIOCHEM BEHAV 31(2) 425–429, 1988.—It has been suggested that supersensitivity to, or overactivity of, endogenous opioid systems, particularly the dynorphin system, may be important in the development of obesity in the obese mouse (ob/ob). We have investigated the possibility that an increase in sensitivity to kappa agonists may also play a role in the development of obesity in another mutant rodent, the Zucker rat. The effects of the selective kappa agonist PD-117302 were investigated in both lean and obese Zucker rats. The lean animals appeared to be more sensitive to the initial hyperphagic effects of PD-117302 than their obese littermates, although this initial hyperphagia was followed by a subsequent decrease in food intake so that by the end of the six-hour test period the animals treated with PD-117302 had eaten less than the saline-treated controls. These changes in food intake were parallelled by increases and decreases in the duration of time spent feeding throughout the experiment. At the doses used in this study PD-117302 had no effect on locomotor activity. It is concluded that obese Zucker rats are not more sensitive to the hyperphagic effects of the kappa agonist PD-117302 than their lean littermates and therefore it seems unlikely that increased sensitivity to an endogenous kappa opioid system plays a major part in the overeating and obesity observed in this strain of rat.

Hyperphagia Zucker rats Kappa agonist PD-117302

HOMOZYGOUS rats of the Zucker strain have a genetic defect which predisposes them to the development of obesity (12). It is believed that the obesity in these animals is at least partly due to an increase in food intake since the obese animals voluntarily eat 50% more than normal lean littermates (10). The neurochemical mechanisms that are responsible for this hyperphagia are not known although it seems likely that several factors are involved. Obese Zucker rats have been reported to be more sensitive to the anorectic effects of the opioid antagonist naloxone than the heterozygous lean animals and it has been suggested that increased sensitivity to and/or increased levels of endogenous opioids may be involved in the aetiology of obesity in this strain of rat (8). Ferguson-Segall and co-workers (3) demonstrated that obese (ob/ob) mice were more sensitive to the hyperphagic effects of the kappa agonists ethylketocyclazocine and Mr2033 than their lean littermates and they suggested that overeating and the development of obesity in these animals is associated with changes in sensitivity to kappa agonists. Since it is not known whether the precise physiological mechanisms that result in the development of obesity in these two different species is the same, it was decided to test the hypothesis that homozygous obese Zucker rats may also be more sensitive to the hyperphagic effects of a kappa agonist than their lean littermates.

PD-117302 was chosen as a suitable kappa agonist for this study in view of its high selectivity for the kappa receptor compared to the mu opiate receptor (1) and also because we have previously shown it to be effective at stimulating food intake in rats of a different strain that did not have a genetic defect predisposing them to the development of obesity (5).

METHOD

Animals

Twelve homozygous (fa/fa) obese Zucker rats (males, starting weight 317±5 grams) and twelve heterozygous (fa/Ola) lean littermates (males, starting weight 222±4 grams), Olac, Bicester, UK, were used in this study. Animals were housed individually for the duration of this study in cages adapted for use in a feeding, drinking and locomotor activity monitor (9). Animals were maintained on a feeding/deprivation schedule as follows: food was available from 10.00 hr to 16.00 hr daily, the food hopper was then removed for the following eighteen hours. Water was available at all times. A period of two weeks was allowed for the animals to become accustomed to eating their normal daily intake of food within this six-hour period before experiments were started. On each experimental day animals were randomly assigned to treatment groups (n=6 per group). Since four groups of animals were used per dose of drug (obese + saline, obese + drug, lean + saline, lean + drug) each exper-

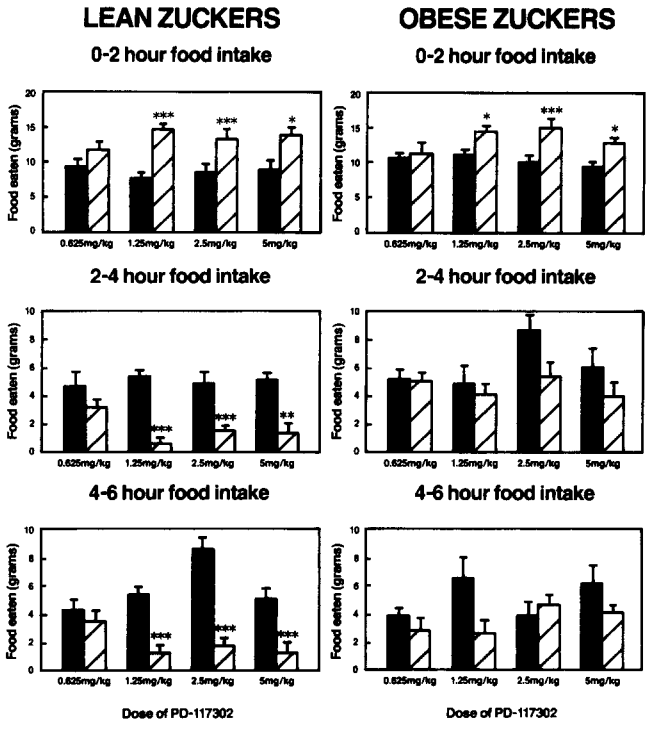

FIG. 1. The effects of PD-117302 (IP) on food intake in the Zucker rat. The three panels on the left hand side of the figure show the effects of increasing doses of PD-117302 in lean animals in comparison with saline-treated controls for the 0–2, 2–4 and 4–6 hour measurement intervals. The right hand part of the figure shows the corresponding results obtained in the obese animals. Results are shown as mean values ±SEM for food intake in grams. n=6 per group. The solid bars represent the saline controls and the hatched bars the PD-117302-treated animals. *$p<0.05$, **$p<0.01$, ***$p<0.005$ significantly different from saline controls.

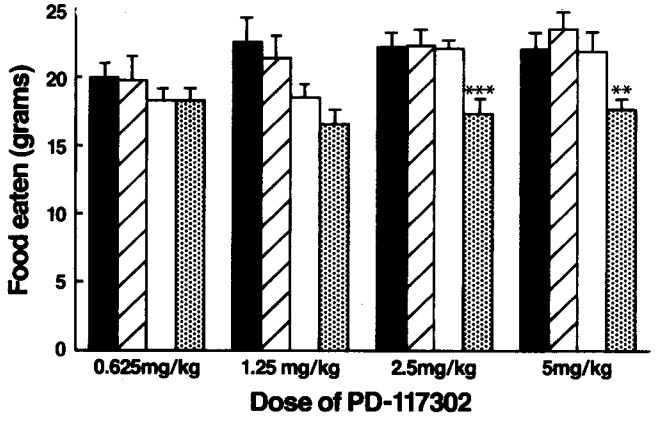

FIG. 3. The effects of PD-117302 on total food intake over the six-hour test period in obese compared with lean Zucker rats. PD-117302 had no effect on overall food intake in the obese animals at any of the doses tested, whereas the higher doses (2.5 and 5.0 mg/kg) produced a statistically significant reduction in total food intake in the lean animals compared to the lean controls. Results are shown as mean±SEM for each group (n=6 per group). *$p<0.05$, ***$p<0.005$ compared to the saline-treated lean controls, Mann-Whitney U-test. The solid bars represent the saline-treated obese animals, the hatched bars the PD-117302-treated obese animals, the open bars the saline-treated lean animals and the stippled bars the PD-117302-treated lean animals.

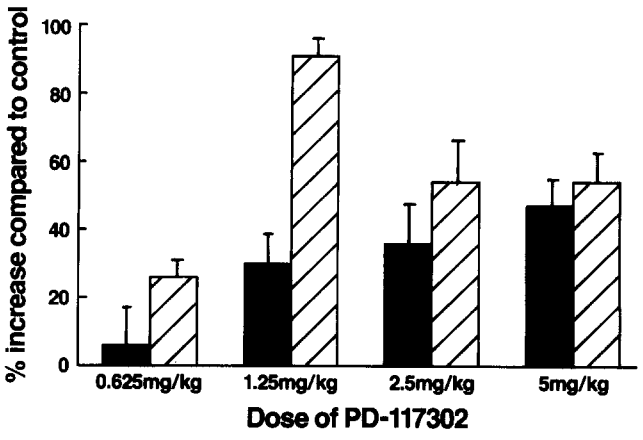

FIG. 2. A summary of the effects of PD-117302 on food intake in lean and obese Zucker rats in the first two hours after dosing, demonstrating the increased hyperphagic response seen in lean (hatched bars) compared to the obese animals (solid bars) at each of the doses of PD-117302 tested. Results are shown as percentage increase in food intake produced by PD-117302 in comparison with the appropriate saline-treated control group (n=6 per group).

iment was split over two days since the feeding, drinking and activity monitor could only hold a maximum of twelve cages. Animals were used repeatedly with a period of at least five drug-free days allowed between experiments. The colony/experimental room was maintained at a constant temperature (21±1°C) and humidity on a fixed twelve hour light, twelve hour dark cycle (lights on 06.00 hr).

Measurement of Food Intake

Animals were dosed subcutaneously five minutes before being presented with the preweighed food hopper containing powdered food (CRM diet, Labsure). The feeding, drinking and activity monitor was activated at this point and used to collect data at five-minute intervals for the entire six hours of the feeding period. Food hoppers were weighed 2, 4, and 6 hours after the start of the experiment.

Statistical Analysis

Statistical comparisons between drug-treated and control groups were made using the Mann-Whitney U-test.

Drugs

PD-117302 (±) trans-N-methyl-N-[2-(1-pyrrolidinyl)-cyclo hexyl] benzo [b]thiophene-4-acetamide hydrochloride (Parke-Davis) was dissolved in saline (0.9% w/v NaCl) and administered in a dose volume of 1 ml/kg.

RESULTS

Effects of PD-117302 on Food Intake

PD-117302 was administered at doses between 0.625 and 5 mg/kg. The minimum dose required to produce a statistically significant change in the amount of food eaten was 1.25 mg/kg. This dose of PD-117302 produced an increase in food intake of 30% in the obese rats compared to the saline-treated controls and 91% in the lean animals compared to the lean controls over the first two hours of the experiment. For

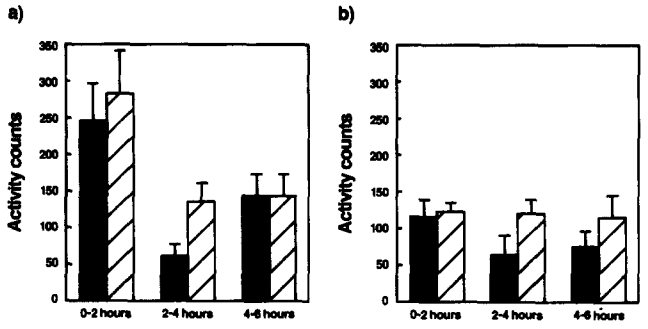

FIG. 4. This figure shows the level of locomotor activity determined in (a) lean, or (b) obese Zucker rats following treatment with either saline (solid bars) or 5.0 mg/kg PD-117302 (hatched bars). The levels of locomotor activity are displayed as values obtained in each of the two-hour time periods over which food intake was measured to allow a direct comparison between the time course of the effects of PD-117302 on food intake and levels of activity. At no time after dosing did either the lean or the obese PD-117302-treated animals show levels of activity that were below the respective saline-treated control groups. Results are shown as mean±SEM for each group, n=6 per group.

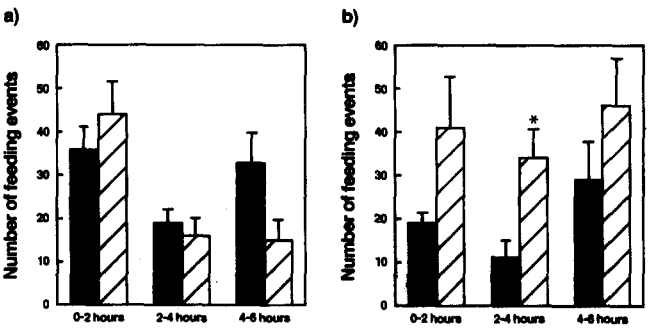

FIG. 5. Number of attendances at the food hopper for (a) lean and (b) obese Zucker rats following treatment with either saline (solid bars) or 5.0 mg/kg PD-117302 (hatched bars). Results are shown as the mean±SEM number of attendances per two-hour data analysis interval. n=6 rats per group. *$p<0.05$ compared to control. This dose of PD-117302 increased the number of attendances at the food hopper by the obese animals but did not affect the number of attendances made by the lean animals.

the remaining four hours of the experiment no significant differences were observed between the amount of food eaten by the obese PD-117302-treated group and the obese controls. The pattern of feeding activity was somewhat different in the lean animals however. During the 2–4-hour measurement interval food intake was reduced by 89% compared to the controls. This reduction in food intake persisted for the following two-hour period with food intake in the PD-117302-treated animals reduced by 76% compared to the amount eaten by the controls.

Similar patterns of changes in food intake were seen with doses of 2.5 and 5 mg/kg PD-117302 as shown in Fig. 1. Figure 2 shows a comparison between the percentage increases in food intake produced by PD-117302 compared to control for both the obese and the lean Zucker rats when food intake was measured over the first two hours of the experiment. At each dose of PD-117302 tested the percentage increase in food intake compared to the controls was greater for the lean than for the obese rats suggesting that the lean rats are more sensitive to the hyperphagic effects of this compound. If, however, the amount of food eaten over the entire six-hour test period is analysed none of the doses of PD-117302 produce a change in the amount of food eaten by the obese Zuckers compared to the saline-treated control group. In the lean animals on the other hand, PD-117302 produced a dose-related reduction in the total amount of food eaten (Fig. 3). This reduction in food intake did not occur as a consequence of sedation since no differences between the levels of locomotor activity were observed between the saline-treated groups and those treated with PD-117302. The results obtained with 5 mg/kg PD-117302 (the highest dose tested) are shown in Fig. 4 as an example. As has been reported by other workers previously (1,3) we observed a marked difference in levels of basal activity in the lean compared to the obese Zucker rats with the obese animals being less active than the lean animals.

Effects of PD-117302 on Feeding Behaviour

The effects of PD-117302 on both the number of attendances at the food hopper and the duration of these attendances were measured. The number of feeding events seen in the obese and lean animals treated either with saline or 5.0 mg/kg PD-117302 are shown in Fig. 5. For the sake of clarity only results obtained with 5.0 mg/kg PD-117302 are shown since the effects of lower doses were essentially similar.

The number of feeding events observed was greater in the lean than in the obese animals and this probably reflects the differences in levels of basal locomotor activity. PD-117302 had no effect on the number of feeding events in the lean Zucker rats compared to the saline-treated controls. In contrast to the lean animals PD-117302 appeared to increase the number of feeding events in the obese animals. This dose of PD-117302 also produced changes in the duration of these feeding events. In the lean animals an increase of 117% compared to the controls was observed during the first two-hour measurement interval. This increase in feeding duration was followed by a reduction in feeding time compared to saline-treated controls which persisted for the remainder of the 6-hour experiment. These increases and decreases in feeding time parallel the effects of this dose of PD-117302 on food intake as do the effects on feeding time in the obese animals. In these animals PD-117302 produced a marked increase in feeding duration in the first two-hour measurement interval only with no differences being observed between the treated and the control groups for the remainder of the experiment.

DISCUSSION

The results obtained in this study suggest that homozygous obese Zucker rats do not display an increased sensitivity to the hyperphagic effects of the kappa agonist PD-117302. It seems instead that the lean Zucker rat is in fact more sensitive than the obese animal to the hyperphagic effect of PD-117302, a highly selective kappa agonist. A recent independent report (6) also provides supporting evidence that the lean Zucker is more sensitive than the obese animal to kappa agonist activity, although in this case it was the diuretic response to the kappa agonist bremazocine that was being evaluated.

The reduction in food intake that follows the initial hyperphagic response to PD-117302 is a phenomenon that we have previously observed in normal Wistar rats (5) following

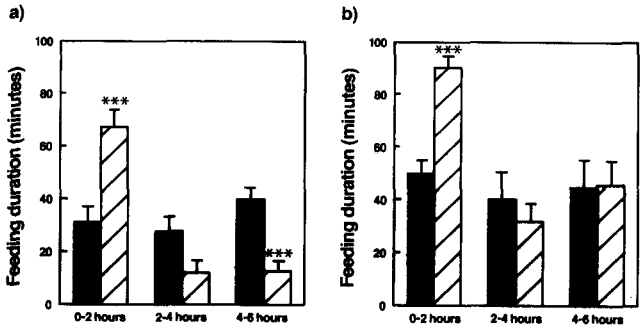

FIG. 6. This figure shows the total amount of time spent feeding during each two hour data analysis interval (i.e., feeding duration) in (a) lean and (b) obese Zucker rats following treatment with either saline (solid bars) or 5.0 mg/kg PD-117302 (hatched bars). Results are shown as the mean±SEM total duration of attendance at the food hopper for each two-hour data analysis interval. n=6 rats per group. ***$p<0.005$ compared to control. PD-117302 produced a significant increase in the lean animals within the first two-hour data analysis interval. This corresponds with the duration of the hyperphagic effect of this compound. In the subsequent four hours of the experiment the time spent feeding was considerably less in the lean PD-117302-treated group compared to the lean controls as would be predicted from the reduced food intake in this group. In the obese animals, in contrast, PD-117302 produced only an initial increase in feeding duration compared to the controls with no subsequent reduction over the remaining four hours.

treatment with either PD-117302 or another kappa agonist, U-50488.

The time course of this effect was somewhat different in the previous study where the animals were feeding freely over a 24-hour test period, with the initial hyperphagic phase of the response to PD-117302 or U-50488 lasting at least six hours and then being followed by a reduction in overnight food consumption. The total amount of food eaten over 24 hours was less in the kappa agonist-treated animals than the respective control groups. It is possible that the difference in the time course of the effect of PD-117302 on feeding behaviour could be explained by differences in the elimination half-life of this compound in the Zucker rat compared to the Wistar rat; unfortunately no data are available to support or contradict this proposition.

It has been suggested (4) that the control of food intake in the genetically obese rat is weaker or less precise than that in the lean rat, and that the control system is accordingly more susceptible to external perturbation and is slower to recover from its effects. This did not seem to be the case in a study where Zucker rats were injected with diazepam since McLaughlin and Baile (7) showed that diazepam was more effective at increasing food intake in lean than in obese Zucker rats. In that study, as in the current series of experiments, the animals were injected with the same dose/kg body weight. As a consequence of this the obese animals received almost twice the dose received by the lean animals. Some authors have argued that obese animals should be dosed according to their lean body weight so that each animal in a lean/obese pair receives the same absolute amount of drug (11). However we felt that since little is known about the precise pharmacokinetics of PD-117302 we would not be justified in dosing our animals in this way. If PD-117302 was selectively accumulated in the brain of these animals and distribution and metabolism were not affected by the large fat depots in the obese Zucker rats then it would be expected that the differences in sensitivity to the effects of PD-117302 between the lean and the obese rats would be even more marked if the doses were adjusted for lean body weights.

In conclusion, it seems that although the lean Zucker rats are more sensitive to the initial hyperphagic effects of the kappa agonist PD-117302 than their obese littermates, this hyperphagic phase is followed by an anorectic effect which overcompensates for the initial increase in food intake so that by the end of the six-hour test period the treated animals have eaten less overall than the saline-treated control group. In the obese animals on the other hand, PD-117302 produced a less marked hyperphagia during the first two hours of the experiment followed in some cases by a slight reduction in food intake over the next four hours so that by the end of the test period the treated animals had eaten the same amount of food as the controls. In the light of these results it seems unlikely to be increased sensitivity to an endogenous kappa opioid system which is responsible for the overeating and obesity observed in this strain of rat. It would perhaps be interesting to study the hyperphagic effects of this compound in animals that were not meal trained but which had free access to food to see whether a similar pattern of hyperphagia followed by a subsequent reduction of food intake is observed.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. D. Horwell and colleagues for the synthesis of PD-117302.

REFERENCES

1. Clark, C. R.; Birchmore, B.; Sharif, N. A.; Hunter, J. C.; Hill, R. G.; Hughes, J. [^{3}H] PD-117302: A selective radioligand for the kappa opioid receptor. Br. J. Pharmacol., in press; 1988.
2. Enns, M. P.; Weker, J.; Grinker, J. Interrelationships among activity, food intake and weight gain in genetically obese and lean Zucker rats. Physiol. Behav. 28:1059–1064; 1982.
3. Ferguson-Segall, M.; Flynn, J. J.; Walker, J.; Margules, D. L. Increased immunoreactive dynorphin and leu-enkephalin in posterior pituitary of obese mice (ob/ob) and supersensitivity to drugs that act at kappa receptors. Life Sci. 31:2233–2236; 1982.
4. Grinker, J. A.; Drenowski, A.; Enns, M.; Kissileff, H. Effects of d-amphetamine and fenfluramine on feeding patterns and activity of obese and lean Zucker rats. Pharmacol. Biochem. Behav. 12:265–275; 1980.
5. Hewson, G.; Hill, R. G.; Hughes, J.; Leighton, G. E.; Turner, W. D. The kappa agonists PD-117302 and U-50488 produce a biphasic effect on 24 hour food intake in the rat. Neuropharmacology 26:1581–1584; 1987.

6. Leander, J. D.; Hart, J. C.; Zerbe, R. L. Kappa agonist induced diuresis: Evidence for stereoselectivity, strain differences, independence of hydration variables and a result of decreased plasma vasopressin levels. J. Pharmacol. Exp. Ther. 242:33–39; 1987.
7. McLaughlin, C. L.; Baile, C. A. Cholecystokinin, amphetamine and diazepam and feeding in lean and obese Zucker rats. Pharmacol. Biochem. Behav. 10:87–93; 1979.
8. McLaughlin, C. L.; Baile, C. A. Feeding behaviour responses of Zucker rats to naloxone. Physiol. Behav. 32:755–761; 1984.
9. Marshall, R. W.; Leighton, G. E.; Hewson, G.; McGarrigle, P.; Bichard, V.; Hill, R. G. A microcomputer system to monitor and analyse locomotion and feeding and drinking behaviours. Br. J. Pharmacol., in press; 1988.
10. Powley, T. L.; Morten, S. A. Hypophysectomy and regulation of body weight in the genetically obese Zucker rat. Am. J. Physiol. 230:982–987; 1976.
11. Roane, D. S.; Porter, J. R. Nociception and opioid induced analgesia in lean (fa/−) and obese (fa/fa) Zucker rats. Physiol. Behav. 38:215–218; 1986.
12. Zucker, L. M.; Zucker, T. F. Fatty—a new mutation in the rat. J. Hered. 52:275–278; 1961.