Pharmacology Biochemistry & Behavior, Vol. 33, pp. 637–640. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

L364,718 Antagonizes the Cholecystokinin-Induced Suppression of Locomotor Activity

J. SOAR, G. HEWSON, G. E. LEIGHTON, R. G. HILL[1] AND J. HUGHES

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

Received 9 January 1989

SOAR, J., G. HEWSON, G. E. LEIGHTON, R. G. HILL AND J. HUGHES. *L364,718 antagonizes the cholecystokinin-induced suppression of locomotor activity.* PHARMACOL BIOCHEM BEHAV **33**(3) 637–640, 1989.—To determine the role of CCK-A receptors in the cholecystokinin (CCK)-induced suppression of locomotor activity in the rat, the ability of the selective CCK-A receptor antagonist L364,718 to block these responses was investigated. Cholecystokinin octapeptide (CCK8) (10, 100 μg/kg IP) and caerulein (1, 5, 10 μg/kg IP) produced marked reductions in locomotor activity whereas cholecystokinin tetrapeptide (CCK4) (100 μg/kg IP) was without effect. The reductions in activity produced by CCK8 (10 μg/kg) and caerulein (10 μg/kg) were antagonized by L364,718 (100 μg/kg IP). In an open field test CCK8 (10 μg/kg IP) reduced locomotor activity and total number of rears and increased pause duration. These effects of CCK8 on open-field behaviour were also antagonized by L364,718 (100 μg/kg IP). It is concluded that L364,718 is a potent antagonist of the actions of CCK8 and caerulein on locomotor activity, suggesting that the effects of these peptides are mediated by a CCK-A receptor.

L364,718 Cholecystokinin Locomotor activity

CHOLECYSTOKININ (CCK) has been reported to reduce food intake and produce the behavioural state of postprandial satiety in a variety of species including sheep, rhesus monkeys, rats and man (17). One component of the postprandial satiety sequence is a reduction in locomotor activity. CCK in the form of the sulphated octapeptide (CCK8) has been reported to reduce exploratory activity in rodents (4). In the rat, both the reductions in food intake and locomotor activity produced by intraperitoneal administration of CCK8 are abolished by destruction of vagal afferent fibres (15,18) suggesting that these effects are initially due to an action in the periphery.

On the basis of receptor binding studies using various fragments of CCK and the use of selective receptor antagonists, it is now accepted that there are 2 types of CCK receptor, namely, CCK-A and CCK-B receptors (1, 2, 11, 16, 18). CCK-A receptors are found mainly in the periphery and mediate effects such as gall bladder contraction and pancreatic amylase secretion (12). CCK-B receptors are found in the CNS where their precise function is unclear. The present study investigated the effects of L364,718 [3S(−)-N-(2,3 dihydro-1-methyl-2-oxo-S-phenyl-1-H-1,4-benzodiazepine-3-yl)-1H indole-2-carboxamide], which is a highly selective and potent antagonist at the CCK-A receptor both in vitro and in vivo (1,14), on the reductions in locomotor activity produced by CCK8 and caerulein. In addition, the ability of L364,718 to antagonize the reduction in exploratory activity produced by CCK8 in an open field was determined. The results show that L364,718 is an effective antagonist of these behavioural effects of CCK8 and caerulein suggesting that they are mediated by an action at CCK-A receptors.

METHOD

Animals

Male Hooded Lister rats (Olac), weighing 200–300 g, were used in all experiments. They were housed in groups of six and maintained on a 12-hour light-dark cycle (lights on 0700 hr) at a constant room temperature of 21 ± 1°C. Lighting levels were subdued during the light phase. CRM pellet food (Labsure) and water was available to rats at all times except during tests.

Drugs

L364,718 (Merck, Sharpe & Dohme) was prepared in 0.3 ml glycerol and 0.7 ml polyethylene glycol 400 per mg of L364,718 and diluted to the required concentration with distilled water. CCK8 (CRB), CCK4 (Sigma) and caerulein (Bachem) were stored as frozen aliquots (corrected for peptide content) and diluted to the required concentration with sterile 0.9% w/v NaCl. All drugs were

[1]Present address: Smith, Kline & French Research Limited, The Frythe, Welwyn, Hertfordshire, AL6 9AR, UK.

injected via the intraperitoneal route using a dose volume of 1 ml/kg.

Experimental Procedures

All testing was carried out in the room in which the animals were housed, between 1100–1800 hr during the light phase of the rats diurnal cycle. Each rat was used only once and was not allowed an habituation period in the test environment prior to testing, in order to maximize the initial period of exploratory behaviour and thus allow any suppression in activity produced by the peptides under investigation to be clearly seen.

Spontaneous locomotor activity studies. Locomotor activity studies were carried out using a computer assisted monitoring system (10), which consisted of a rack of 12 perspex test cages (internal dimensions $20 \times 42 \times 20$ cm). The long axis of each cage was bisected by a single infrared beam and an activity count was registered each time a rat passed through this beam. After the beam was broken, another activity count could only be registered once the beam was reestablished.

In order to investigate the effects of peptides on spontaneous locomotor activity, groups of ten rats were injected with CCK8 (1, 10, 100 μg/kg), caerulein (1, 5, 10 μg/kg), CCK4 (100 μg/kg) or vehicle, placed individually into test cages and the collection of activity data started.

In the L364,718 interaction studies, rats were pretreated with L364,718 (100 μg/kg) or vehicle and returned to their home cages. Thirty minutes after pretreatment, rats were injected with CCK8 (10 μg/kg), caerulein (10 μg/kg) or vehicle, placed individually into test cages and the monitoring of locomotor activity begun. The dose and pretreatment time used for L364,718 were the same as that found to be effective at antagonising the reduction in food intake produced by CCK8 (6).

In the above experiments, locomotor activity counts were collected in 5-minute bins over a period of 20 minutes. A test period of 20 minutes was chosen as animals in the control groups reached basal activity levels after this time.

Open-field behaviour studies. These studies were carried out using an open-field apparatus which consisted of a square floor area (60×60 cm) enclosed by 22.5 cm high walls made from opaque white perspex. The square floor area of the open field was divided by red lines into a grid of nine squares (20×20 cm each). The following behavioural activities were scored: 1) Locomotor activity, defined as the total number of line crossing by both front and rear paws; 2) Total number of rears, defined as the number of vertical extensions of head, body and forelimbs, either free standing, or against a wall; 3) Total pause duration, defined as the total time spent stationary.

In this study rats were randomly assigned to treatment groups and pretreated with either L364,718 (100 μg/kg IP) or vehicle 30 minutes before receiving either CCK8 (10 μg/kg IP) or vehicle. Rats were returned to their home cages until they were tested in the open field 5 minutes after the second injection. At the start of each test, a rat was placed in the centre square of the grid and behavioural events were scored over the 5-minute test sessions by an observer uninformed of the drug treatments.

Statistical Analysis

Statistical comparisons between treatment groups and the appropriate controls were made by using the Kruskal-Wallis one-way ANOVA followed by the Mann-Whitney U-test (two-tailed). Significance was accepted for $p<0.05$.

RESULTS

Spontaneous Activity Studies

CCK8 markedly reduced locomotor activity at doses of 10 μg/kg and 100 μg/kg when compared to the vehicle-treated control group (Fig. 1). A lower dose of 1 μg/kg CCK8 failed to produce any significant change in locomotor activity. Caerulein also markedly reduced activity at all doses tested, whereas CCK4 was without effect (Fig. 2).

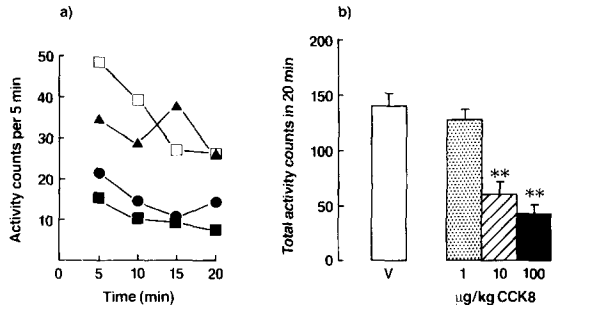

FIG. 1. CCK8 produces a reduction in locomotor activity. (a) Time course over the 20-min test period. Each point represents the mean activity counts per 5 min. □ Vehicle; ▲ 1 μg/kg; ● 10 μg/kg; ■ 100 μg/kg CCK8. (b) Total activity over the 20-min test period. Each bar represents the mean total activity counts (+SEM) (V = vehicle). $**p<0.01$ significantly different from the vehicle-treated group Mann-Whitney U-test (two-tailed) following a significant Kruskal-Wallis one-way ANOVA ($p<0.01$). n = 10 rats per treatment group.

In the drug-interaction studies, CCK8 (10 μg/kg IP) produced a significant reduction in locomotor activity when compared to the control (vehicle + vehicle) group. L364,718 significantly antagonized the reduction in locomotor activity produced by CCK8 (Fig. 3). The reduction in locomotor activity produced by caerulein (10 μg/kg IP) was also attenuated by L364,718 (Fig. 4), although the L364,718 + caerulein group showed a slight, but significant, reduction compared to the control group. In neither experiment did the locomotor activity of the groups treated with L364,718 + vehicle differ from the control groups.

Open-Field Behaviour Studies

In the open field, CCK8 (10 μg/kg IP) produced significant reductions in the total number of line crossings and in the total number of rears, with a concomitant increase in total pause

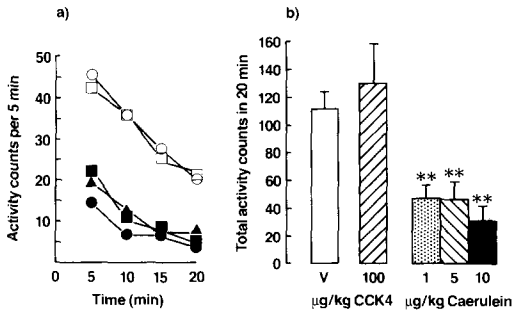

FIG. 2. The effect of caerulein and CCK4 on locomotor activity. (a) Time course over the 20 min test period. Each point represents the mean activity counts per 5 min. □ Vehicle; ○ 100 μg/kg CCK4; ■ 1 μg/kg; ▲ 5 μg/kg; ● 10 μg/kg caerulein. (b) Total activity over the 20-min test period. Each bar represents the mean total activity counts (+SEM) (V = vehicle). $**p<0.01$ significantly different from the vehicle-treated group Mann-Whitney U-test (two-tailed) following a significant Kruskal-Wallis one-way ANOVA ($p<0.01$). n = 10 rats per treatment group.

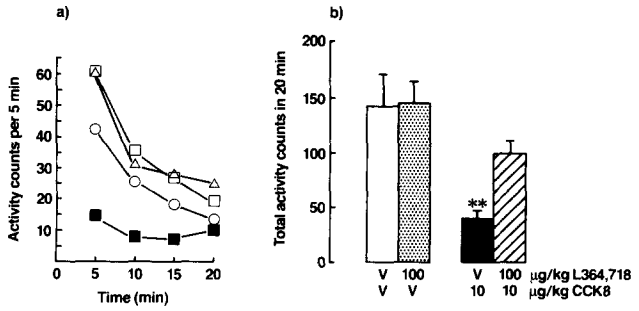

FIG. 3. L364,718 (100 µg/kg IP) antagonizes the reduction in locomotor activity produced by CCK8 (10 µg/kg IP). (a) Time course over the 20-min test period. Each point represents the mean activity counts per 5 min. □ Vehicle + vehicle; △ L364,718 + vehicle; ■ vehicle + CCK8; ○ L364,718 + CCK8. (b) Total activity over the 20-min test period. Each bar represents the mean total activity counts (+SEM) (V = vehicle). $^{**}p<0.01$ significantly different from the vehicle + vehicle-treated group Mann-Whitney U-test (two-tailed) following a significant Kruskal-Wallis one-way ANOVA ($p<0.01$). n = 10 rats per treatment group.

duration. The effects of CCK8 on these three behavioural parameters were completely antagonized by L364,718 pretreatment (Fig. 5). L364,718 alone did not affect the total number of rears observed or the pause duration, but did produce a small increase in the total number of line crossings when compared to the control group.

DISCUSSION

CCK8 and caerulein produced a marked reduction in locomotor activity in the present study, in agreement with a number of previous reports (4, 5, 7, 13, 15). Although full dose-response relationships were not established, it is clear that caerulein reduced locomotor activity at a lower dose than CCK8. The greater potency of caerulein compared to CCK8 with regard to a number of their biological effects is well documented (6, 20, 21).

The results of the present study demonstrate a clear antagonism by L364,718 of the reduction in locomotor activity produced by CCK8 and caerulein in the rat. L364,718 shows good selectivity

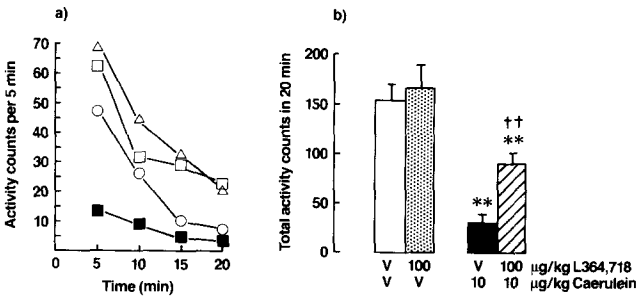

FIG. 4. L364,718 (100 µg/kg IP) antagonizes the reduction in locomotor activity produced by caerulein (10 µg/kg IP). (a) Time course over the 20-min test period. Each point represents the mean activity counts per 5 min. □ Vehicle + vehicle; △ L364,718 + vehicle; ■ vehicle + caerulein; ○ L364,718 + caerulein. (b) Total activity over the 20-min test period. Each bar represents the mean total activity counts (+SEM) (V = vehicle). $^{**}p<0.01$ significantly different from vehicle + vehicle-treated group; $^{††}p<0.01$ significantly different from vehicle + caerulein-treated group Mann-Whitney U-test (two-tailed) after significant Kruskal-Wallis one-way ANOVA ($p<0.01$). n = 10 rats per treatment group.

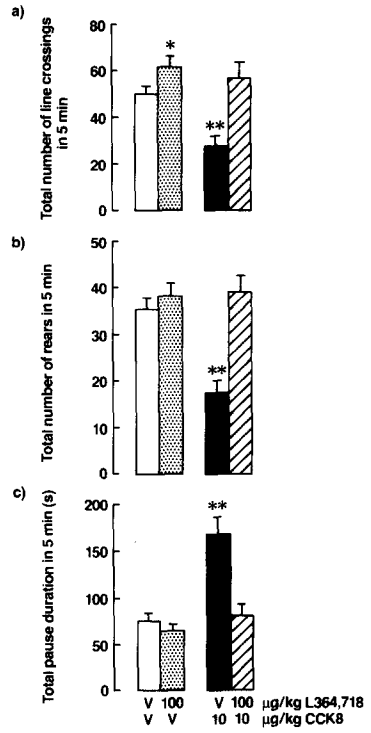

FIG. 5. L364,718 antagonizes the effects of CCK8 on open-field behaviour. Rats were pretreated with L364,718 (100 µg/kg IP) or vehicle 30 min before receiving either CCK8 (10 µg/kg IP) or vehicle. Individual rats were placed in the open field 5 min after the second injection and the following behaviours quantified: (a) total number of line crossings; (b) total number of rears; (c) total pause duration (sec). Bars represent the mean total activity (+SEM) during the 5-min test sessions (V = vehicle). $^{*}p<0.05$; $^{**}p<0.01$ significantly different from the vehicle + vehicle-treated group Mann-Whitney U-test (two-tailed) following a significant Kruskal-Wallis one-way ANOVA ($p<0.01$). n = 8 rats per treatment group.

for CCK-A receptors in vitro (1). It also antagonized the locomotor effects at a similar dose level as that reported to antagonize other in vivo effects of CCK8 thought to be mediated by CCK-A receptors (1,14). Thus, the present results suggest that the reduction in locomotor activity produced by CCK8 and caerulein was mediated by an action at CCK-A receptors.

The observation that CCK receptor blockade antagonises the reduction in locomotor activity produced by CCK is in agreement with earlier studies which showed that the CCK antagonists benzotript and proglumide blocked the reduction in exploratory activity produced by CCK8 (5). These antagonists show weak activity at CCK-A receptors, however, and differentiate poorly between CCK-A and CCK-B receptors (3,19). Recently, L364,718 has been reported to antagonize the increase in pause duration produced by CCK8 in an open-field test in mice (8). The results obtained in the present study in the rat agree with this finding and show that L364,718 also antagonizes the effects of CCK8 on line crossing and rearing in the open field.

CCK4 failed to have any effect on spontaneous locomotor activity on peripheral administration when given at a large dose relative to the doses of CCK8 and caerulein which produced reductions in locomotor activity. CCK4 has a relatively high affinity for CCK-B receptors, but negligible affinity for CCK-A receptors (2,16). Thus, if CCK-B receptors were involved in the reduction in locomotor activity produced by CCK8 and caerulein,

the dose of CCK4 used might have been expected to produce a similar effect. The lack of effect of CCK4, therefore, provides some additional evidence that CCK-A receptors are involved in the locomotor effects of peripherally-administered CCK. The lack of effect of CCK4 in this study is in agreement with results showing that high doses of CCK4 have no effect on locomotor activity after SC administration in mice (13).

L364,718 had no effect on locomotor activity measured in the automated monitor system, but increased locomotor activity slightly in the open field. It is possible that this is a reflection of the greater sensitivity of the latter to detect such changes when compared to testing in a cage bisected by a single light beam. It is unclear whether the slight increase in locomotor activity represents a true effect of the drug, however. The results obtained in a study carried out in mice reported that L364,718 had no effect on pause duration (8).

The present study did not address the location of the CCK-A receptors involved in the reduction in locomotor activity produced by CCK. Studies which have reported on the site of action of peripherally-administered CCK provide evidence that the initial effect on locomotor activity is in the periphery and mediated by the vagal afferent pathway (7,15). It is therefore likely that the CCK-A receptors at which caerulein and CCK8 act to reduce locomotor activity are in the periphery and associated in some way with the vagal afferent pathway.

It is concluded that the reduction in locomotor activity produced by CCK8 and caerulein was mediated by activation of CCK-A receptors.

REFERENCES

1. Chang, R. S. L.; Lotti, V. J. Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. Proc. Natl. Acad. Sci. USA 83:4923–4926; 1986.
2. Clark, C. R.; Daum, P.; Hughes, J. A study of the cerebral cortex cholecystokinin receptor using two radiolabelled probes: Evidence for a common CCK 8 and CCK 4 cholecystokinin receptor binding site. J. Neurochem. 46:1094–1101; 1986.
3. Clark, C. R.; Daum, P.; Hughes, J. Lack of competition between two reputed peripheral cholecystokinin receptor antagonists for central cholecystokinin binding sites. Ann. NY Acad. Sci. 448:581–582; 1985.
4. Crawley, J. N. Divergent effects of cholecystokinin, bombesin, and lithium on rat exploratory behaviors. Peptides 4:405–410; 1983.
5. Crawley, J. N.; Stivers, J. A.; Hommer, D. W.; Skirboll, L. R.; Paul, S. M. Antagonists of central and peripheral behavioral actions of cholecystokinin octapeptide. J. Pharmacol. Exp. Ther. 236:320–330; 1986.
6. Hewson, G.; Leighton, G. E.; Hill, R. G.; Hughes, J. The cholecystokinin receptor antagonist L364,718 increases food intake in the rat by attenuation of the action of endogenous cholecystokinin. Br. J. Pharmacol. 93:79–84; 1988.
7. Houpt, T. R.; Anika, S. M.; Wolff, N. C. Satiety effects of cholecystokinin and caerulein in rabbits. Am. J. Physiol. 235: R23–R28; 1978.
8. Itoh, S.; Katsuura, G. Behavioral effect of cholecystokinin octapeptide in vagotomized rats. Can. J. Physiol. Pharmacol. 64:745–747; 1986.
9. Khosla, S.; Crawley, J. N. Potency of L-364,718 as an antagonist of the behavioral effects of peripherally administered cholecystokinin. Life Sci. 42:153–159; 1988.
10. Makovec, F.; Bani, M.; Chiste, R.; Revel, L.; Rovati, L. C.; Rovati, L. A. Differentiation of central and periperal cholecystokinin receptors by new glutaramic acid derivatives with cholecystokinin-antagonist activity. Arzneimittelforschung 36:98–102; 1986.
11. Marshall, R. W.; Sparks, M. G.; Davies, J. A. A microcomputer for long-term monitoring and analysis of locomotor activity. In: Redfern, P. H.; Campbell, I. C.; Davies, J. A.; Martin, K. F., eds. Circadian rhythms in the central nervous system. London: Macmillan; 1985: 263–270.
12. Moran, T. H.; Robinson, P. H.; Goldrich, M. S.; McHugh, P. R. Two brain cholecystokinin receptors: implications for behavioural actions. Brain Res. 362:175–179; 1986.
13. Morley, J. E. The ascent of cholecystokinin from gut to brain. Life Sci. 30:479–493;1982.
14. Moroji, T.; Hagino, Y. A behavioral pharmacological study on CCK-8 related peptides in mice. Neuropeptides 8:273–286; 1986.
15. Lotti, V. J.; Pendleton, R. G.; Gould, R. J.; Hanson, H. M.; Chang, R. S. L.; Clineschmidt, B. V. In vivo pharmacology of L-364,718, a new, potent nonpeptide peripheral cholecystokinin antagonist. J. Pharmacol. Exp. Ther. 241:103–109; 1987.
16. Ritter, R. C.; Kalivas, P.; Bernier, S. Cholecystokinin-induced suppression of locomotion is attenuated in capsaicin pretreated rats. Peptides 7:587–590; 1986.
17. Sakamoto, C.; Williams, J. A.; Goldfine, I. D. Brain CCK receptors are structurally distinct from pancreas CCK receptors. Biochem. Biophys. Res. Commun. 124:497–502;1984.
18. Smith, G. P.; Gibbs, J.; Jerome, C.; Pi-Sunyer, F. X.; Kissileff, H. R.; Thornton, J. The satiety effect of cholecystokinin: A progress report. Peptides 2(Suppl. 2):57–59; 1981.
19. Smith, G. P.; Jerome, C.; Norgren, R. Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. Am. J. Physiol. 249:R638–R641; 1985.
20. Vigna, S. R.; Szecowka, J.; Williams, J. A. Do antagonists of pancreatic cholecystokinin receptors interact with central nervous system cholecystokinin receptors? Brain Res. 343:394–397; 1985.
21. Zetler, G. Analgesia and ptosis caused by caerulein and cholecystokinin octapeptide (CCK-8). Neuropharmacology 19:415–422; 1980.
22. Zetler, G. Caerulein and cholecystokinin octapeptide (CCK-8): sedative and anticonvulsant effects in mice unaffected by the benzodiazepine antagonist Ro 15-1788. Neurosci. Lett. 28:287–290; 1982.