Psychopharmacology (1985) 86:281–285

Psychopharmacology

Aversive properties of naloxone in non-dependent (naive) rats may involve blockade of central β-endorphin

Ronald F. Mucha, Mark J. Millan, and Albert Herz
Department of Neuropharmacology, Max-Planck-Institut für Psychiatrie, Am Klopferspitz 18a, D-8033 Planegg-Martinsried, FRG

Abstract. The present study examines the influence of destruction of the medio-basal arcuate hypothalamus (MBH), the primary site of synthesis of central pools of β-endorphin (β-EP), upon the aversive properties of naloxone in a conditioned place preference paradigm. Bilateral radiofrequency lesions of the MBH resulted in a pronounced fall in levels of immunoreactive β-EP in the brain. Lesioned rats, in contrast to non-operated animals, showed a clear reduction in the conditioned place aversion produced by naloxone. However, they showed no loss of the conditioned preference produced by the mu-selective opioid receptor agonist, morphine, or the conditioned aversion produced by the kappa-selective agonist, U50-488. In contrast to the effect of the lesions, suppression of circulating β-EP by dexamethasone treatment failed to influence conditioning produced by naloxone. Thus, the data indicate that the aversive properties of naloxone are attenuated by disruption of central (but not peripheral) β-EP activity. We suggest that these properties of naloxone reflect an antagonism of β-EP activity in the brain. In addition, the data indicate that differing mechanisms underlie the aversive actions of naloxone as compared to U50-488.

Key words: Conditioned place preference – Aversions – Naloxone – Morphine –U50-488 – β-endorphin – Dynorphin – Arcuate nucleus – Rat

Although competitive antagonists such as naloxone are highly aversive in morphine-dependent animals (Downs and Woods 1976; Goldberg et al. 1971), only recently, largely through the development of sensitive preference conditioning procedures, has it proven possible to reveal that naloxone is also aversive in non-dependent animals. Thus, the aversive effects of naloxone have been seen with different species (monkey and rat) and in a range of models (classically conditioned taste and place preferences and operantly conditioned responding; Downs and Woods 1976; Mucha et al. 1982; Stolerman et al. 1978). In addition, these effects of naloxone both occur at low doses and are stereospecific (Mucha et al. 1982; Stolerman et al. 1978), reflecting the drug's potent activity on opioid receptors.

Offprint requests to: R.F. Mucha

Since naloxone exhibits little or no intrinsic agonist activity on opioid receptors (Kosterlitz and Watt 1968), its aversive effects are probably due to antagonism of the action of an endogenous opioid upon these receptors (Downs and Woods 1976; Mucha et al. 1982; Stolerman et al. 1978). However, the identity of this endogenous opioid is not presently known. In fact, there are three families of endogenous opioid peptides corresponding to three precursor molecules: pro-opiomelanocortin, pro-enkephalin A, and pro-enkephalin B. Major gene products of these precursors are β-endorphin (β-EP), met-enkephalin, and dynorphin A (DYN), respectively (Höllt 1983). The central β-EP network, the cell bodies of which are primarily located in the medio-basal arcuate hypothalamus (MBH) (Millan et al. 1980; Watson et al. 1978), is of special interest in the present context. Van Ree et al. (1979) have demonstrated that rats self-administer β-EP into the cerebral ventricles, and it has been shown that β-EP levels in the hypothalamus are altered in response to cues paired with reward (Dum et al. 1982). Moreover, β-EP terminals are found in many areas of the brain suspected to be involved in processes of motivation (Finley et al. 1981; Mogenson et al. 1980), and microinjections of opioids into these areas, such as the nucleus accumbens, periacquedeuctal gray, and ventral tegmental area, produce conditioned place preferences (Phillips and LePiane 1980; Van der Kooy et al. 1982). Finally, naloxone is an effective blocker of the pharmacological effects of β-EP (Huidobro-Toro and Way 1979; Wüster et al. 1980).

Therefore, this study addressed the hypothesis that naloxone may produce its aversive effects via a blockade of the actions of β-EP-containing neurons in the brain. It is known that lesions in the region of MBH result in severe damage to β-EP-containing neurones in the brain (Millan et al. 1980; Watson et al. 1978). Accordingly, such lesions should affect the conditioning produced by naloxone. Since morphine acts directly on opioid receptors as an agonist, its appetitive reinforcing properties should not, in contrast, be affected.

In addition, the present approach allowed for a comparison between the conditioning produced by naloxone and that by a kappa-receptor agonist. There is evidence to suggest that kappa-receptor agonists can act as antagonists of β-EP and morphine-like drugs (Gillan et al. 1982; Petrillo et al. 1984; Wüster et al. 1980). Thus, if the aversive effects of naloxone and kappa agonists likewise reflect an antago-

nistic action, they should be similarly sensitive to particular manipulations such as lesions of the MBH.

Materials and methods

The subjects used were male Sprague-Dawley rats (Iwanowas, Kissleg, F.R.G.) weighing 180–220 g. They were housed in groups of three to six.

Place conditioning was carried out employing apparatus and procedures described earlier (Mucha and Herz 1985). Briefly, it involved the differential pairing of drug and vehicle with two sets of place cues: one comprised a black box with a smooth black floor and the other a white box with a textured white floor. The procedure is an "unbiased" method, as with the particular apparatus and testing conditions rats do not show an overall bias for one or the other of the sets of environmental cues (Mucha and Iversen 1984; Mucha and Herz 1985). Thus, without any pretesting, each rat was placed for 1 h in a different box twice a day for 3 consecutive days, once in the morning (0800–1200 hours) and once, at least 6 h later, in the late afternoon or early evening (1600–2000 hours). For one half of the animals in an experiment, drug was administered just before the rat was placed in the black box and saline just before placement in the white box, or vice versa; assignment to the particular group was random. Testing was then carried out on the day following completion of training. Rats were placed individually without any drug for 15 min into a rectangular testbox constructed to give a rat a choice between the two sets of place cues: one set was at each end separated by a small neutral grid platform. During the test period, a video camera was used to determine how much time the rat spent on the drug- and on the saline-paired sides of the testbox. Only one drug was examined in an individual rat.

In each experiment lesions of the MBH were carried out 7 days prior to conditioning. As described previously (Millan et al. 1980, 1984), the lesions were bilateral, carried out with standard stereotaxic technique with the following coordinates (according to König and Klippel 1963): 4.3 mm rostral, 0.4 mm dorsal, and ± 0.6 mm lateral to interaural zero. The radiofrequency electrode was lowered to the appropriate position and the tip temperature maintained at 55° C for 25 s. The sham-lesioned rats received identical treatment except that the tip was 1.4 mm dorsal and the electrode was not activated. To confirm that these surgical procedures themselves had no effect, in a preliminary experiment it was demonstrated that sham-operated rats did not differ from control animals as regards their ability to show naloxone-produced place aversions.

Treatment with dexamethasone was carried out by substitution of normal drinking water for water containing 20 µg/ml dexamethasone 60 h prior to the first conditioning trial (Höllt et al. 1981). The drug was maintained in the drinking fluid until the rats were killed.

Two days after testing, rats were decapitated. The brain was then removed and dissected on ice for estimation of levels of immunoreactive (ir) β-EP and irDYN in hypothalamus, midbrain, and anterior pituitary. The procedures employed for dissection, extraction, radioimmunoassay, and evaluation of properties of the antisera have been described previously (cf. Millan et al. 1984; Weber et al. 1982). Briefly, the β-EP antiserum used recognized β-EP and β-lipotropin to an equimolar extent, but did not cross react with α- or β-neo-endorphin, leu-enkephalin, BAM-related peptides, met-enkephalin, or DYN. The antiserum to DYN (generously provided by E. Weber) did not cross-react with α- or β-neo-endorphin, BAM-related peptides, β-EP, leu-enkephalin, met-enkephalin, or dynorphin$_{1-8}$.

In addition to biochemical verification of the efficacy of destruction of the β-EP neurons, in order to evaluate the location of the lesions, certain rats were examined histologically. These animals were killed and their brains were removed. Sections (20 µ) were than obtained on a cryostat and stained with toluidine blue.

The drug solutions were prepared with physiological saline and injected SC in a volume of 1 ml/kg. Maximal doses were used for conditioning, derived from dose-response curves determined by use of the same apparatus, protocol and population of rats as used here (Mucha and Herz 1985). They were expressed as the free base.

The basic datum of the conditioning was the amount of time spent by each rat on the drug-paired side of the testbox minus the amount of time spent on the vehicle side (a negative value indicates an aversion). The data are presented as means $\pm$ SEM. Differences were evluated using two-tailed Student's t-test.

Results

Lesioned rats displayed a pronounced depletion of irβ-EP in the hypothalamus and midbrain reflecting destruction of central β-EP-containing neurones, as exemplified by the data from the naloxone experiments (Fig. 1). It was previously shown that the lesions do not affect irβ-EP levels in the systemic circulation (Millan et al. 1980, 1984). Further, the lesioned rats showed a fall in irDYN in the hypothalamus in line with previous work (Millan et al. 1984), though the tendency for a decrease in the midbrain did not attain statistical significance (Fig. 1). We previously demonstrated that such lesions do *not* significantly modify immunoreactive met-enkephalin levels in hypothalamus or other brain areas (Millan et al. 1984). The histological analysis confirmed that the site of the destruction was in the region of the arcuate nucleus of the hypothalamus. The exact site of such lesions has been extensively described previously (Millan et al. 1980).

The lesioned rats, in contrast to the sham group, showed significantly less conditioned place aversion (Fig. 2), but they still retained a marginal response to naloxone as seen by the fact that their aversions just reached the criterion of significance ($t=2.19$, $df=17$, $P<0.05$). The rats were in excellent health and during conditioning their behaviour appeared similar to that of the shams.

In contrast, conditioned place preferences produced by morphine (1 mg/kg) were unaffected by the lesions (Fig. 2), although the location of the lesion and the pattern of the depletions were similar to those described above. Irβ-EP in hypothalamus of sham and lesion rats was 22.4 ± 2.4 ($n=9$) and 5.0 ± 0.6 fmole/mg ($n=13$), respectively.

Similarly, conditioned place aversions produced by the κ receptor ligand, U50-488 (1 mg/kg), were not changed significantly by the lesions (Fig. 2). The levels of irβ-EP in the hypothalamus of these sham (16.4 ± 2.3, $n=8$) and lesion rats (4.0 ± 0.8 fmole/mg, $n=16$) indicated that the lesions were comparable to those of the naloxone and morphine experiments.

Glucocorticoids are known to suppress plasma and pi-

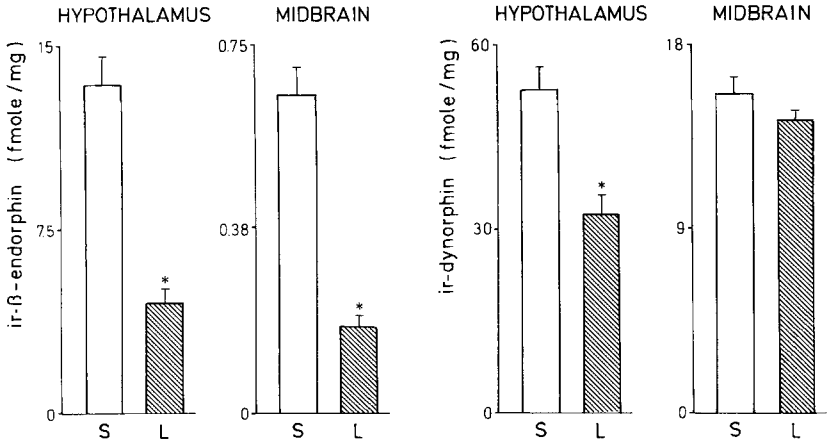

Fig. 1. Levels of immunoreactive β-endorphin and dynorphin in hypothalamus and midbrain in rats which received bilateral radiofrequency lesions of the hypothalamic arcuate nucleus (*L*, $n=18$) or sham lesions (*S*, $n=16$). The data were from the rats of the naloxone experiment. The *asterisk* denotes a significant difference from control (at least $P<0.01$)

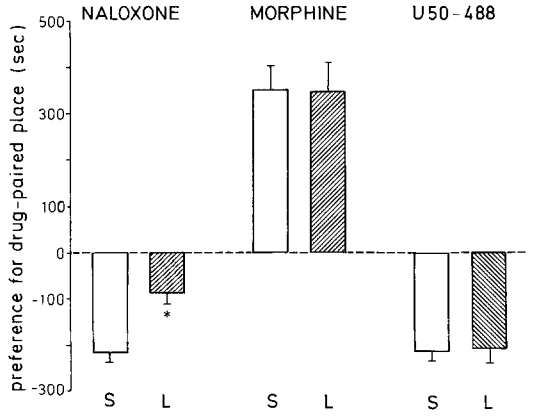

Fig. 2. Preference for drug-paired place (mean difference between time spent on drug and saline side of the testbox) of lesioned (*L*) and sham (*S*) rats. The data were from different experiments in which different rats received place conditioning with 1 mg/kg naloxone (*left panel*), morphine (*middle*), or U50-488 (*right panel*). The means comprise at least eight nonlesioned rats and at least 12 lesioned ones. The *Asterisk* refers to a significant difference between lesioned and sham rats ($P<0.02$)

Table 1. Content of irβ-endorphin in anterior lobe of pituitary and place conditioning with naloxone (1 mg/kg) in rats given normal water as the drinking fluid ($n=11$) or water containing 20 μg/ml dexamethasone ($n=12$). *Asterisk* denotes significant difference from control ($P<0.01$)

Drinking fluid	Irβ-endorphin content of anterior pituitary (pmole/lobe)	Preference for naloxone place (s)
Water	319±31.8	−230±51
Dexamethasone	127±5.1*	−210±46

tuitary levels of β-EP (Guillemin et al. 1977): therefore such a manipulation was used to test whether changes in circulating irβ-EP affect conditioning with naloxone. To confirm the efficacy of the dexamethasone treatment (Höllt et al. 1981), we evaluated the anterior pituitary content of irβ-EP (Table 1) which was, as anticipated, decreased. However, the dexamethasone produced no effect on the place aversion produced by naloxone (1 mg/kg) (Table 1).

Discussion

The present study demonstrates that bilateral destruction of the MBH results in an attenuation of the conditioned place aversion produced by the opioid antagonist, naloxone, in the rat. In contrast, the lesions did not affect the conditioned place preference produced by morphine, a mu-selective opioid receptor agonist, or conditioned place aversions produced by U50-488, a kappa-selective opioid receptor agonist (von Voigtlander et al. 1983). The persistence of the place preference and aversion to, respectively, morphine and U50-488 in lesioned rats is of note. It indicates, thus, the selectivity of the interference with the aversion to naloxone, and demonstrates that the rats do *not* suffer from any generalized disruption of learning processes or other mechanisms which might have resulted in an inability to acquire a conditioned place response. Indeed, in a previous study we have established that rats with lesions of the MBH show no differences from unoperated counterparts as regards their gross behaviour or, for example, daily food intake and exploratory behaviour (Millan et al. submitted).

The MBH is the primary site for synthesis of central β-EP-containing neurones, the perikayra of which are located within this region. Destruction of MBH, consequently, severely damaged the central network of β-EP-containing neurones resulting in the pronounced depletion in levels of irβ-EP detected both in the hypothalamus itself and in a major projection target, the midbrain. Since naloxone's aversive effects appear to result from the interruption of the activity of an endogenous opioid (see Introduction), this damage to central β-EP neurones may underlie the blockade produced by the lesions of the aversive qualities of naloxone. Thus, we suggest naloxone acts via an antagonism of central β-EP transmission. Such a possibility is entirely consistent with the fact that actions of β-EP are readily blocked by naloxone and also the anatomical, biochemical, and pharmacological data indicative of a relationship between β-EP and motivational processess (see Introduction). Further, the present observation that suppression of circulating levels of ir-β-EP by dexamethasone treatment fails to modify the place aversion conditioning produced by naloxone, together with the fact that MBH lesions do not modify irβ-EP levels in the systemic circulation, is indicative of the specificity of this effect to central as compared to hypophyseal-derived peripheral pools of β-EP.

Nevertheless, it must be recognized that the effects of

lesions of MBH are not restricted to elimination of central β-EP-containing neurones and there are two alternative explanations of the diminished aversive affects of naloxone in lesioned rats.

Firstly, it might be argued that the lesions destroy sites to which naloxone binds (in the MBH) in the production of its aversive effect. However, the possibility that the sites are in the arcuate nucleus is unlikely since this area, a circumventricular region, lies external to the blood brain barrier (cf. Olney et al. 1977) and work with systemic application of quaternary naltrexone, which does not penetrate the blood brain barrier, suggests that conditioning of aversion with opioid antagonists requires passage through this barrier (Bechara and van der Kooy 1984). In addition if it can be assumed that morphine produces its appetitive reinforcing effect at receptor sites in common with those at which naloxone produces aversive effects, since conditioning produced by morphine is not affected by the lesions, naloxone is presumably not acting at the site of destruction. The maintained response to morphine is also of importance in indicating that the influence of the lesions does not reflect interruption of a network downstream of the relevant opioid receptor population involved in the expression of opioid agonist or antagonist effects in motivational processes.

Secondly, it must be considered whether the reduction of conditioning with naloxone might reflect the depression in hypothalamic levels of ir-DYN precipitated by the lesions. This would, however, appear to be unlikely for the following reasons. Thus, naloxone only poorly antagonizes DYN-produced pharmacological effects and binds only weakly to kappa receptors, which are believed to be the sites of DYN activity in the brain (Chavkin et al. 1982; Han and Xie 1984; Robson et al. 1983; Wüster et al. 1981). In addition agonists selective for κ-receptors produce only conditioned aversions (Mucha and Herz 1985): accordingly, interruption of DYN activity with naloxone would *not* be expected to produce an aversion.

Recent evidence suggests that synthetic agonists selective for the kappa opioid receptor can also act as antagonists at other opioid receptors (see Introduction). Therefore, their aversive effects and those of naloxone could in theory be due to a common mechanism, i.e. an antagonism of the activity of an endogenous opioid. However, the present data indicate that the conditioned aversion produced by κ-agonists is unlikely to reflect a blockade of central β-EP activity, since, in distinction to the effect of naloxone, the place aversion produced by the κ-agonist, U50-448, was *not* disrupted by destruction of central β-EP neurones.

To summarize, we suggest that the aversive properties of the opioid antagonist naloxone primarily reflects an antagonism of the activity of central β-EP-containing neurones. Additional experiments employing, for example, selective antibodies against β-EP may prove instructive in the further evaluation of this hypothesis. Whether the residual aversion seen in MBH-lesioned rats reflects sub-total β-EP elimination or an action of naloxone on a further system cannot presently be specified. In addition, the data reveal that contrasting mechanisms underlie the aversive effects of naloxone as compared to those of a κ-agonist.

Acknowledgements. R. Mucha was supported by the Alexander von Humboldt Stiftung (Bonn) and the Bundesgesundheitsamt (Berlin). M.J. Millan was supported by the Deutsche Forschungsgemeinschaft (Bonn). R.F. Mucha is now at the following address: Behavioural Pharmacology, Addiction Research Foundation, Toronto, Ontario,M55 2S1.

References

Bechara A, van der Kooy D (1984) Endogenous opioids: opposite motivational effects in brain and periphery. Neurosci Soc Abst 10:312

Chavkin C, James IF, Goldstein A (1982) Dynorphin is a specific endogenous ligand of the κ opioid receptor. Science 215:413–415

Downs DA, Woods JA (1976) Naloxone as a negative reinforcer in rhesus monkeys: effect of dose, schedule, and narcotic regimen. Pharmacol Rev 27:397–436

Dum J, Gramsch C, Herz A (1983) Activation of hypothalamic β-endorphin pools by reward induced by highly palatable food. Pharmacol Biochem Behav 18:443–457

Finley JCW, Lindström P, Petrusc P (1981) Immunocytochemical localization of β-endorphin-containing neurons in the rat brain. Neuroendocrinology 33:28–42

Gillan MGC, Kosterlitz HW, Magnan J (1981) Unexpected antagonism in the rat vas deferens by benzomorphans which are agonist in other pharmacological tests. Br J Pharmacol 72:13–15

Goldberg SR, Hoffmeister F, Schlichting V, Wuttke W (1971) Aversive properties of nalorphine and naloxone in morphine-dependent monkeys. J Pharmacol Exp Ther 179:268–276

Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, Bloom F (1977) β-endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. Science 197:1367–1369

Han JS, Xie GX (1984) Dynorphin: important mediator for electroacupuncture in the spinal cord of the rabbit. Pain 18:367–376

Höllt V (1983) Multiple endogenous opioid peptides. Trends Neurosci 6:24–26

Höllt V, Haarmann I, Seizinger BR, Herz A (1981) Levels of dynorphin (1–13) immunoreactivity in rat neurointermediate pituitaries are concomitantly altered with those of leucine-enkephalin and vasopressin in response to various endocrine manipulations. Neuroendocrinology 33:333–339

Huidobro-Tori JP, Way EL (1979) Studies on the hypothermic response of β-endorphin in mice. J Pharmacol Exp Ther 211:50–58

König JFR, Klippel RA (1963) The rat brain (a stereotaxic atlas). Williams and Wilkins, Baltimore

Kosterlitz HW, Watt AJ (1968) Kinetic parameters of narcotic agonists and antagonists, with particular reference to N-allylnoroxymorphone (naloxone). Br J Pharmacol 33:266–276

Millan MH, Millan MJ, Przewłocki R (1984) Lesions of the hypothalamic arcuate nucleus modify discrete brain and pituitary pools of dynorphin in addition to β-endorphin in the rat. Neurosci Lett 48:149–154

Millan MJ, Gramsch C, Przewłocki R, Höllt V, Herz A (1980) Lesions of the hypothalamic arcuate nucleus produce a temporary hyperalgesia and attenuate stress-evoked analgesia. Life Sci 27:1513–1523

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69–97

Mucha RF, Herz A (1985) Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology (in press)

Mucha RF, Iversen LL (1984) Reinforcing properties of morphine and naloxone revealed by conditioned place preferences, a procedural examination. Psychopharmacology 82:244–247

Mucha RF, van der Kooy D, O'Shaughnessy M, Bucenieks P (1982) Drug reinforcement studied by use of place conditioning in rat. Brain Res 243:91–105

Olney JW, Rhee V, de Gubarett T (1977) Neurotoxic effects of glutamate on mouse area postrema. Brain Res 120:151–157

Petrillo P, Gambino MC, Tavani A (1984) Bremazocine induces antinociception, but prevents opioid-induced constipation and catatonia in rats and precipitates withdrawal in morphine-dependent rats. Life Sci 35:917–927

Phillips AG, LePiane FG (1980) Reinforcing effects of morphine microinjection into the ventral tegmental area. Pharmacol Biochem Behav 12:965–968

Robson LE, Paterson SJ, Kosterlitz HW (1983) Opiate receptors. In: Iversen LL, Iversen SD, Snyder SH (eds) Handbook of psychopharmacology Vol 17, Plenum Press, New York, pp 13–80

Stolerman I, Pilcher CW, D'Mello GD (1978) Stereospecific aversive property of narcotic antagonists in morphine-free rats. Life Sci 22:1755–1762

van der Kooy D, Mucha RF, O'Shaughnessy M, Bucenieks P (1982) Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. Brain Res 243:107–117

van Ree JM, Smyth DG, Colpaert FC (1979) Dependence creating properties of lipotropin C-fragment (β-endorphin): evidence for its internal control of behavior. Life Sci 24:195–202

von Voigtlander PE, Lahti RA, Ludens JH (1983) U50, 488: A selective and structurally novel non-mu (kappa)opioid agonist. J Pharmacol Exp Ther 224:7–12

Watson SJ, Akil H, Richard CW, Barchas JD (1978) Evidence for two separate opiate peptide neuronal systems. Nature 275:226–228

Weber E, Evans CJ, Barchas JD (1982) Predominance of the amino-terminal octapeptide fragment of dynorphin in rat brain regions. Nature 299:77–79

Wüster M, Schulz R, Herz A (1980) Opioid agonists and antagonists. Action on multiple opiate receptors. In: Way LL (ed) Endogenous and exogenous opioid agonists and antagonists. Pergamon Press, New York, pp 75–78

Wüster M, Schulz R, Herz A (1981) Multiple opiate receptor in peripheral tissue preparations. Biochem Pharmacol 30: 1883–1887

Received December 11, 1984; Final version March 6, 1985