Br. J. Pharmacol. (1989), **98**, 903–913

Differential cardiovascular and respiratory responses to central administration of selective opioid agonists in conscious rabbits: correlation with receptor distribution

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1 The effects of intracerebroventricular (i.c.v.) and intracisternal (i.c.) administration of a range of doses (0.01, 0.1 and 1.0 nmol kg^{-1}) of specific μ- δ- and κ-opioid agonists on cardiovascular and respiratory function and on plasma catecholamines have been studied in conscious rabbits. The distribution of μ- δ- and κ-opioid receptors was localized in rabbit brain by *in vitro* autoradiography.

2 The μ-agonist [D-Ala^2, MePhe^4-Gly^5-ol]enkephalin (DAGOL) given i.c.v. caused a large rise in plasma noradrenaline and adrenaline, hypertension accompanied by an initial bradycardia followed by tachycardia, respiratory depression and sedation. After i.c. administration there were similar changes in heart rate (HR) and respiration, but no significant changes in mean arterial pressure (MAP) or plasma catecholamines.

3 The δ-agonist [D-Pen^{2,5}]enkephalin (DPDPE) increased MAP and HR after both i.c.v. and i.c. administration, caused a small increase in noradrenaline but had no effect on adrenaline and did not alter respiration rate or blood gases. After i.c.v. DPDPE the rabbits became more alert and active.

4 The κ-agonist U69593 given i.c.v. or i.c. had no effect on MAP or HR. After i.c.v. U69593, $PaCO_2$ fell, but there were no other respiratory effects. The responses to dynorphin 1-13, an endogenous κ-agonist, were similar to those of U69593.

5 The opioid antagonist naloxone (30 nmol kg^{-1}) given intravenously (i.v.) blocked the effects of i.c.v. DAGOL (1 nmol kg^{-1}). A 100 fold higher dose of i.v. naloxone (3 μmol kg^{-1}) was required to abolish the effects of i.c.v. DPDPE (1 nmol kg^{-1}).

6 Autoradiographic studies demonstrated a high density of μ- and δ-opioid receptors in hypothalamic sites. In the brainstem μ-receptors were demonstrated in the nucleus tractus solitarius (NTS) and δ-receptors in the dorsal motor nucleus of the vagus. κ-Receptors were not detected in either the hypothalamus or brainstem.

7 These findings demonstrate that DAGOL increases sympatho-adrenal outflow, probably by stimulation of hypothalamic μ-receptors. The effects on HR are probably partly through a baroreflex and partly through an action of DAGOL on μ-receptors in the dorsal motor nucleus of the vagus. DPDPE probably acts on δ-receptors in the NTS to increase MAP and HR. Respiratory depression resulted from stimulation of μ-receptors in the brainstem with no evidence of δ- or κ-receptors being involved.

Introduction

A potential role for endogenous opioid peptides in central cardiovascular control has been indicated by the demonstration that opioid peptides and their receptors are present in areas of brain with established roles in the regulation of cardiovascular function (Hokfelt *et al.*, 1977; Goodman *et al.*, 1980). Further evidence came from studies in which central administration of opioid peptides was shown to alter

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sympathetic outflow (Van Loon et al., 1981; Pfeiffer et al., 1983) or to influence baroreflex mechanisms (Schaz et al., 1980). In conscious rabbits we have demonstrated that the predominantly μ-opiate agonist morphine acts centrally to increase sympatho-adrenal outflow and produce hypertension (May et al., 1988; 1989a). Similar responses have been described in conscious rats after intracerebroventricular (i.c.v.) injection or hypothalamic microinjections of the selective μ-opioid agonist [D-Ala^2, MePhe^4, Gly^5-ol] enkephalin (DAGOL) (Pfeiffer et al., 1982; 1983). The cardiovascular effects resulting from stimulation of central δ- and κ-receptors are less clear because of the relative lack of specificity of the agonists that have been available.

In the present study the cardiovascular and respiratory effects of the μ-opioid agonist DAGOL have been compared with the effects of the δ-opioid agonist (D-Pen^{2,5})enkephalin (DPDPE) and the κ-opioid agonist, U69593. The specificity of DPDPE and U69593 for their respective receptor subtypes is an order of magnitude greater than that of other agonists selective for these receptors (Mosberg et al., 1983; Lahti et al., 1985). These selective agonists have been given i.c.v. and intracisternally (i.c.) to differentiate between periventricular and brainstem sites of action. Conscious animals have been used to avoid interference from anaesthetic agents. The possible sites of action have been investigated further by use of *in vitro* autoradiography to localize μ-, δ- and κ-opioid receptor distribution in rabbit brain with the same selective agonists.

Methods

Autoradiography

The *in vitro* autoradiographic technique was essentially that described by Young & Kuhar (1979), modified according to the protocol described by Dashwood et al. (1988). Rabbits were anaesthetized with alphaxalone 0.9%/alphadolone acetate 0.3% (Saffan, Glaxovet) (1 ml i.v. followed by 0.25 ml i.v. as required). They were perfused with 1.0 litre ice cold 100 mM phosphate buffer, pH 7.4, containing 0.32 M sucrose and then lightly fixed with 1.0 litre 0.1% formaldehyde solution in phosphate sucrose buffer (Dashwood et al., 1985). The brains were removed and frozen at -70°C. Frozen sections (20 μm) of brainstem and forebrain were cut serially in a cryostat at -20°C and thaw-mounted onto gelatinised microscope slides. The presence of opioid receptors was established by use of the appropriate tritiated ligands (Gillan & Kosterlitz, 1982; Lahti et al., 1985; Gulya et al., 1985; Dashwood et al., 1985; 1986; 1988). All sections were preincubated in 170 mM Tris HCl buffer, pH 7.4, containing 100 mM NaCl to reduce levels of endogenous transmitter. Generalised opiate binding was established by incubating sections for 60 min at 4°C in 170 mM Tris/100 mM NaCl buffer, pH 7.4, containing 4 nM [^{3}H]-naloxone. The degree of binding to non-specific sites was established by incubating in the presence of 1 μM unlabelled naloxone or morphine. Binding sites of opioid subtypes were identified by incubating in 50 mM Tris/HCl, pH 7.4, containing 5 mM $CaCl_2$, 2 mg ml^{-1} bovine serum albumin, and 20 μg ml^{-1} bacitracin containing 4 nM [^{3}H]-DPDPE, [^{3}H]-DAGOL or [^{3}H]-ethylketocyclazocine (EKC) for 60 min at 22°C. Binding of [^{3}H]-EKC to δ- and μ-sites was prevented by incubating sections in the presence of 100 nM concentrations of DPDPE and DAGOL respectively. Binding to κ-sites was further investigated by incubating sections in 5 nM [^{3}H]-U69593 in 50 mM HEPES buffer, pH 7.4, containing 10 mM $MgCl_2$. The degree of non-specific binding of [^{3}H]-DAGOL, [^{3}H]-DPDPE, [^{3}H]-EKC and [^{3}H]-dynorphin was established by incubation in the presence of 1 and 10 μM unlabelled naloxone. After incubation, sections were washed (three times for 10 min) in buffer minus bacitracin and blown dry in a stream of cold air. Following drying the slide-mounted sections were affixed to 24 × 30 cm X-ray cassettes with double sided tape and apposed to tritium-sensitive film (Hyperfilm ^{3}H, Amersham) for 4 to 12 weeks at 4°C. After this exposure period the films were developed in Kodak D19 developer (undiluted for 5 min at 22°C), fixed in Ilfospeed fixer (diluted 1 to 4 with distilled water for 5 min at 22°C), then rinsed in running tap water for at least 20 min before allowing the films to dry. Autoradiographs were visually examined and, where appropriate, photographed.

In vivo *experiments*

Experiments were carried out on male Sandy Half-Lop rabbits (National Institute for Medical Research, Mill Hill, London) weighing between 2.1 and 3.3 kg; they were allowed free access to food (RHM R14, Labsure Animal Diets) and water.

Surgical procedures

For injection into the right lateral ventricle rabbits were anaesthetized with Saffan (1 ml i.v. followed by 0.25 ml as required) and the cannulae (Harvard Apparatus) were implanted aseptically. Coordinates were 1.0 mm caudal to bregma, 3.0 mm lateral to midline and 8.5 mm below the dura. The opioid agonists were injected i.c.v. in a volume of 25 μl using a Hamilton syringe with a 25 G needle that protruded 1 mm from the cannula tip. The injection was

flushed in with 25 μl of saline with a needle that reached half way down the cannula shaft. For injection into the cisterna magna the catheter described by Head *et al.* (1983) was modified as previously described (May *et al.*, 1989b) and inserted under Saffan anaesthesia (1 ml i.v. followed by 0.25 ml i.v. as required). Injections were given in 25 μl of saline using a Hamilton syringe with a 30 G needle. The injection was flushed in with 20 μl of saline; the dead volume of the catheter was 10 μl.

The injection site was confirmed at the end of a series of experiments by injection of 25 μl of bromophenol blue (1%) either i.c.v. or i.c. followed by the appropriate volume of saline. The animals were killed, the brains removed and the distribution of dye examined.

Experimental protocol

Animals were not used for at least 7 days post operation. Before each experiment cannulae were inserted into the central artery and marginal vein of the ear under 1% lignocaine local anaesthesia. The arterial cannula was connected to a pressure transducer (Bell and Howell) and BP and HR were recorded on a Devices polygraph. During the experiment the rabbits were lightly restrained as previously described (May *et al.*, 1988). Intervals of at least 7 days were allowed between studies.

All agonists were filtered through a sterile 0.2 μm filter before injection from a sterilized Hamilton syringe. After a 30 min control period increasing doses (0.01, 0.1 and 1.0 nmol kg^{-1}) of the agonists were given at 1 h intervals either i.c.v. or i.c. in conscious rabbits. For dynorphin an additional dose of 5.0 nmol kg^{-1} was also given. Naloxone was given via the marginal vein of the ear 15 min after DAGOL and 10 min after DPDPE.

Blood collection

Blood (2 ml) collected from the arterial cannula into fluoride oxalate tubes was centrifuged and glucose was measured in the plasma by a glucose oxidase method using a Chem-Lab analyser. Catecholamines were measured in plasma obtained from blood (2.5 ml) collected into cooled tubes containing 50 μl of EGTA (0.095%)/glutathione (0.06%) and kept on ice until centrifugation at 4°C and then stored at −70°C. Catecholamines were separated by high performance liquid chromatography (h.p.l.c.) and detected by electrochemical detection (May *et al.*, 1988). Immediately following blood collection an equal volume of 0.9% saline was infused i.v. Arterial blood (100 μl) was collected into a capillary tube for the immediate measurement of blood gases (Instrument Laboratory 230 blood gas analyser).

Drugs and chemicals

DAGOL and DPDPE were purchased from Bachem, porcine dynorphin 1-13 from Cambridge Research Biochemicals, 5α, 7α, 8β-(−)-N-methyl-N-(7-(1-pyrrolidinyl)-1 oxaspiro(4,5)dec-8-yl) benzeneacetamide (U69593) from Amersham International and naloxone HCl from Sigma and Endo Labs. DAGOL, DPDPE and naloxone were dissolved in sterile non-pyrogenic isotonic saline (saline). U69593 (5 mg) was dissolved in 35% ethanol in saline (2 ml) and dynorphin (1 mg) was dissolved in 0.002 M acetic acid (1 ml) and further dilutions of both agonists were made in saline.

[^{3}H]-naloxone (42–60 Ci $mmol^{-1}$), [^{3}H]-DPDPE (26–41 Ci $mmol^{-1}$), [^{3}H]-DAGOL (58 Ci $mmol^{-1}$), [^{3}H]-U69593 (40 Ci $mmol^{-1}$) were purchased from Amersham International and [^{3}H]-EKC (24 Ci $mmol^{-1}$) from New England Nuclear.

Statistical analysis

Data are presented as means ± s.e.mean. Analysis was performed on the differences from the control values using the SAS statistical programme. The control values for mean arterial pressure (MAP) and HR consisted of the mean of the six readings taken during the control period. A logarithmic transformation was performed on the values for plasma glucose, noradrenaline and adrenaline before analysis. The data were subjected to analysis of variance and where the null hypothesis was rejected Bonferroni's comparisons were performed on the means; $P < 0.05$ was considered significant.

Results

Autoradiography

In rabbit forebrain and medulla oblongata (shown diagrammatically in Figure 1) there was a striking variation in the distribution of opioid receptor subtypes. With all ligands the degree of non-specific binding was similar to that shown for [^{3}H]-naloxone (Figures 2a and 3a) and in every case it was less than 30% of the total binding. In rabbit forebrain the presence of [^{3}H]-naloxone binding in areas surrounding the 3rd ventricle demonstrated the presence of opioid binding sites in the hypothalamus (Figure 2a). There was also a high density of [^{3}H]-DAGOL and [^{3}H]-DPDPE binding in hypothalamic sites (Figures 2e and 2f) but no binding of [^{3}H]-U69593 (Figure 2d) demonstrating the presence of μ- and δ-opioid binding sites, but the absence of κ-binding sites in the hypothalamus. The low density of [^{3}H]-EKC binding in the hypothalamus (Figure 2c) may

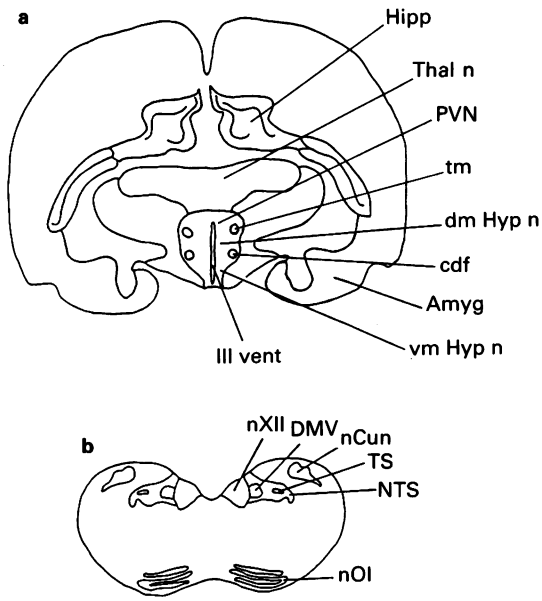

Figure 1 Line drawings of rabbit forebrain (a), and medulla oblongata (b). Abbreviations: (a) Hipp, hippocampus; Thal n, thalamic nuclei; PVN, paraventricular nucleus; tm, tractus mamillothalamicus; dm Hyp n, dorsomedial hypothalamic nuclei; cdf, columna decendens fornicis; Amygd, amygdala; vm Hyp n, ventromedial hypothalamic nuclei; III vent, third ventricle. (b) nXII, hypoglossal nucleus; DMV, dorsomedial nucleus of the vagus; nCun, cuneate nucleus; TS, tractus solitarius; NTS, nucleus of the tractus solitarius; nOl olivary nuclei.

reflect some small degree of binding to μ- or δ-receptors or to a subtype of κ-receptors to which [^{3}H]-U69593 does not bind. There were low levels of [^{3}H]-DAGOL binding in the central amygdaloid nucleus with high levels in the basolateral and medial amygdaloid nuclei (Figure 2e); there was binding of [^{3}H]-DPDPE in all these nuclei (Figure 2f) but there was no binding of [^{3}H]-U69593 (Figure 2d). In addition, there was a high density of μ-binding sites in ventral and dorsal thalamic nuclei and of κ-binding sites in dorsal and lateral thalamic nuclei.

In the brainstem [^{3}H]-DAGOL binding demonstrated the presence of μ-binding sites in the hypoglossal nucleus and the dorsal motor nucleus of the vagus (Figure 3e), whereas [^{3}H]-DPDPE binding indicated the presence of δ-binding sites in the nucleus tractus solitarius (NTS) (Figure 3f). An absence of κ-binding sites in the brainstem is indicated by the lack of [^{3}H]-U69593 or [^{3}H]-EKC binding (Figures 3c and d).

Cardiovascular effects

The μ-opioid agonist DAGOL given i.c.v. caused a dose-related increase in MAP and a fall in HR in six conscious rabbits (Figure 4). There were no changes after the lowest dose, but after 0.1 and 1.0 nmol kg^{-1} there were significant increases in MAP ($P < 0.05$) and falls in HR ($P < 0.05$) within 5 min. At 120 min after the highest dose, MAP had returned to control but HR was significantly elevated above control ($P < 0.05$). Following i.c. administration of the same doses of DAGOL ($n = 5$) there was a trend for MAP to increase and HR to fall but these changes did not reach statistical significance.

The δ-receptor agonist, DPDPE, given i.c.v. to six rabbits caused a dose-related increase in BP which was accompanied by a rise in HR (Figure 5). Following the highest dose (1.0 nmol kg^{-1}) there were significant increases in MAP ($P < 0.05$) and HR ($P < 0.05$) reaching a peak after 15 min. Both MAP and HR fell over the next hour but then increased so that 120 min after the highest dose they were both significantly elevated above control ($P < 0.05$). The same doses of DPDPE given i.c. ($n = 6$) caused similar changes in MAP and HR (Figure 5).

The κ-agonist, U69593, given i.c.v. ($n = 6$) or i.c. ($n = 5$) had no significant effects on MAP or HR at any of the doses used. To confirm that the lack of cardiovascular response to U69593 was not a result of its binding to a subpopulation of κ-receptors, the effects of dynorphin 1-13, an endogenous κ-agonist were examined. Dynorphin given i.c.v. ($n = 4$) or i.c. ($n = 4$) to conscious rabbits had no effect on MAP but after the two highest doses there was a gradual increase in HR which began after 0.1 nmol kg^{-1} and reached 67 ± 20 beats min^{-1} above control ($P < 0.05$) at 60 min after 5.0 nmol kg^{-1}, i.c.

Effects on plasma catecholamines and plasma glucose

Samples for plasma catecholamines were taken at the end of the control period, immediately before the highest dose (60 min after the middle dose) and then at 5, 30, and 60 min after the highest dose.

Following i.c.v. DAGOL (1.0 nmol kg^{-1}) plasma noradrenaline increased 4 fold ($P < 0.05$) and plasma adrenaline 120 fold ($P < 0.05$) and they remained significantly elevated after 60 min (Table 1). After i.c. DAGOL there were no significant increases in noradrenaline or adrenaline. The changes in plasma glucose after i.c.v. and i.c. DAGOL correlated with the changes in adrenaline (Table 1).

Following DPDPE (1.0 nmol kg^{-1}) there was a trend for noradrenaline to increase after i.c.v. and i.c. administration which was significant 60 min after i.c. administration (Table 1). There were no significant increases in plasma adrenaline or glucose.

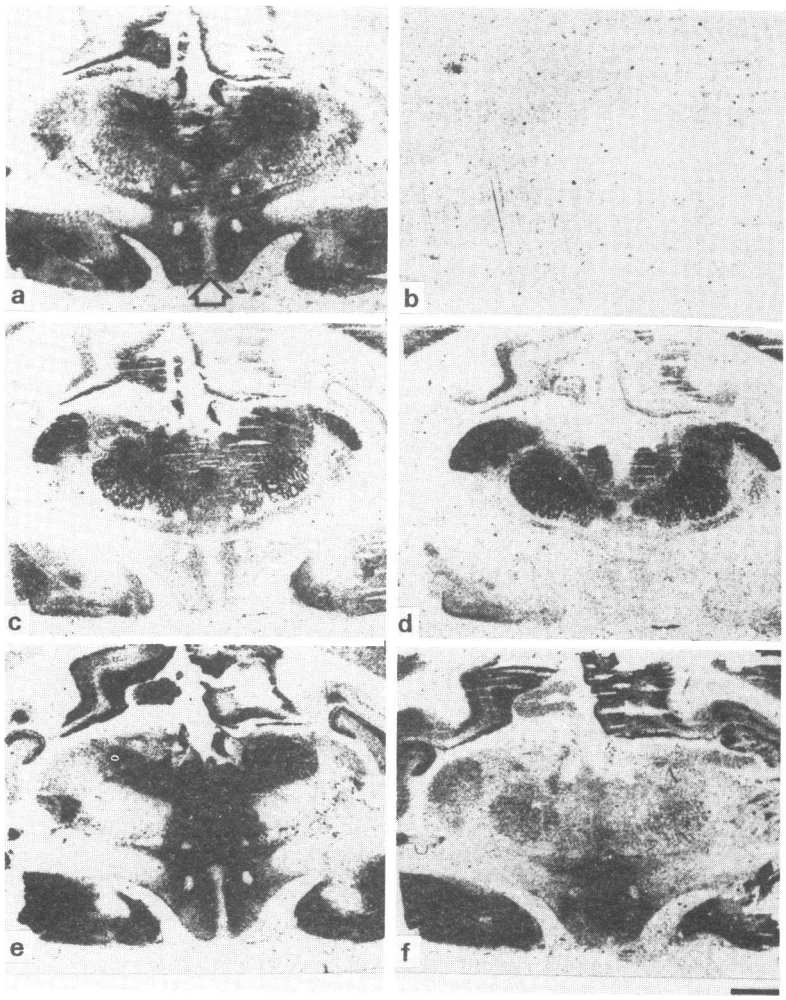

Figure 2 Distribution of opioid receptor subtypes in rabbit forebrain. Consecutive 20 μm sections were incubated with [^{3}H]-naloxone (a), [^{3}H]-naloxone + 1 μM unlabelled naloxone (b), [^{3}H]-ethylketocyclazocine ([^{3}H]-EKC) (c), [^{3}H]-U69593 (d), [^{3}H]-[D-Ala^2, MePhe^4-Gly^5-ol]enkephalin ([^{3}H]-DAGOL) (e) and [^{3}H]-[D-Pen^{2,5}]enkephalin ([^{3}H]-DPDPE) (f). The darker the image the greater the density of receptors. Arrow indicates 3rd ventricle. Scale bar = 2 mm.

U69593 caused no significant changes in noradrenaline after any of the doses given i.c.v. or i.c. There was a small increase in adrenaline of $1.1 \pm 0.5\,\text{nmol}\,l^{-1}$ ($P < 0.05$) at 5 min, but this was not accompanied by a change in plasma glucose. Dynorphin had no effect on plasma noradrenaline or adrenaline levels after i.c.v. or i.c. administration of any of the doses. Plasma glucose was significantly increased ($P < 0.05$) at 30 and 60 min after dynorphin ($5\,\text{nmol}\,l^{-1}$) given i.c.v. (1.1 ± 0.3 and $1.6 \pm 0.2\,\text{mmol}\,l^{-1}$, respectively) and i.c. (1.6 ± 0.7 and $2.0 \pm 0.3\,\text{mmol}\,l^{-1}$, respectively).

Respiratory changes

DAGOL given i.c.v. ($n = 6$) or i.c. ($n = 5$) had no significant effects on respiration rate. After i.c.v. and i.c. DAGOL there was a dose-related increase in $P_{a}CO_2$ and fall in $P_{a}O_2$ (Figure 6). These changes had not developed 5 min after administration when the cardiovascular responses were greatest, but developed slowly over 30 min and began to return to control after 60–90 min. In contrast, DPDPE given i.c.v. ($n = 6$) or i.c. ($n = 6$) had no effects on respiration rate or blood gases. After i.c.v. U69593 ($n = 6$),

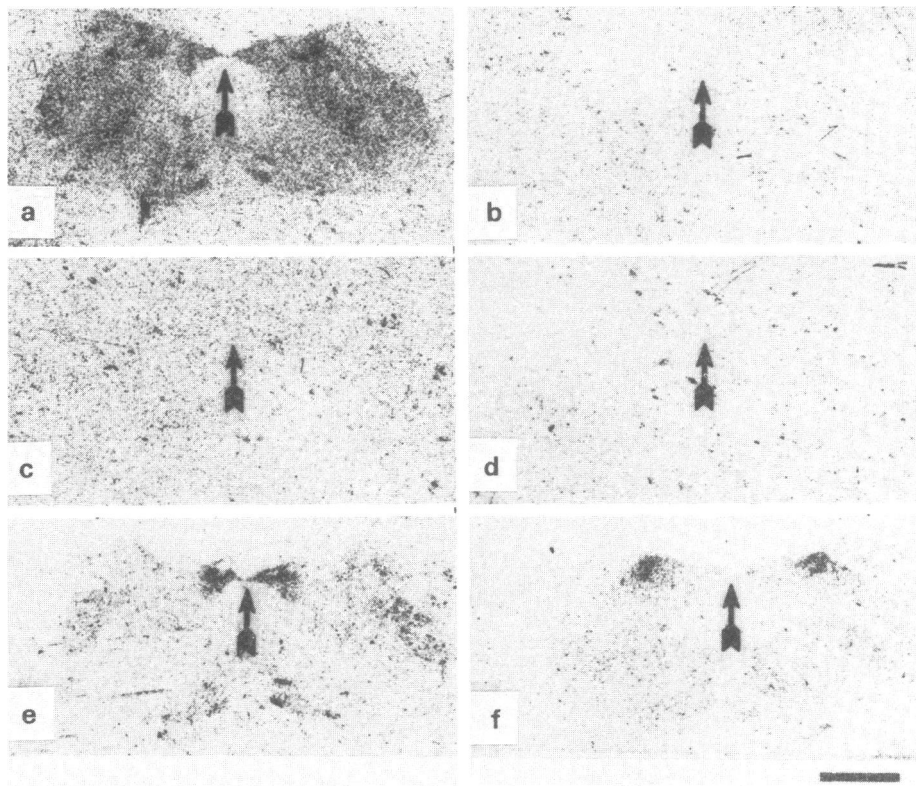

Figure 3 Distribution of opioid receptor subtypes in rabbit medulla oblongata. Consecutive 20 μm sections were incubated with [^{3}H]-naloxone (a), [^{3}H]-naloxone + 1 μM unlabelled naloxone (b), [^{3}H]-EKC (c), [^{3}H]-U69593 (d), [^{3}H]-DAGOL (e) and [^{3}H]-DPDPE (f). The darker the image the greater the density of receptors. Arrow indicates floor of 4th ventricle. Scale bar = 2 mm. Abbreviations as in Figure 2.

$Paco_2$ fell from 30.2 ± 0.7 to 24.9 ± 1.2 mmHg ($P < 0.05$) and remained significantly reduced for 60 min. There were similar falls in $Paco_2$ after i.c.v. dynorphin ($n = 4$). At 15 min after dynorphin, 1.0 nmol kg^{-1}, $Paco_2$ had fallen from 35.4 ± 0.9 to 30.2 ± 1.3 mmHg ($P < 0.05$) and by 60 min after 5.0 nmol kg^{-1}, $Paco_2$ had fallen to 27.5 ± 1.7 mmHg ($P < 0.05$). Neither U69593 nor dynorphin given i.c.v. altered respiration rate or Pao_2. There were no changes in respiration rate or blood gases after any of the doses of i.c. U69593 ($n = 5$) or i.c. dynorphin ($n = 4$).

Behaviour

DAGOL given i.c.v. had a dose-related sedative effect; the rabbits were mildly sedated after the middle dose and heavily sedated for 1–2 h after the highest dose. There was only a mild degree of sedation after the highest i.c. dose. Following i.c.v. but not i.c. DPDPE, the rabbits became more alert and active and started grooming. There were no behavioural changes after U69593 or dynorphin given i.c.v. or i.c.

Effect of i.v. naloxone on responses to i.c.v. DAGOL and i.c.v. DPDPE

DAGOL (1.0 nmol kg^{-1}) administered i.c.v. to six conscious rabbits caused similar changes in MAP, HR, plasma catecholamines and respiration to those seen previously. At 15 min after DAGOL, i.v. administration of naloxone (30 nmol kg^{-1}) caused a significant fall in MAP ($P < 0.05$) and increase in HR ($P < 0.05$) within 5 min (Figure 7); i.v. naloxone (3 nmol kg^{-1}) had no effect. The increase in plasma adrenaline of 22.7 ± 3.0 nmol l^{-1}, 15 min after DAGOL was significantly reduced ($P < 0.05$) within 5 min of naloxone to 4.2 ± 2.1 nmol l^{-1}, whereas the increase in noradrenaline of 3.3 ± 0.4 nmol l^{-1} was not significantly reduced at 5 min after naloxone (1.5 ± 0.3 nmol l^{-1}). In contrast, in the absence of

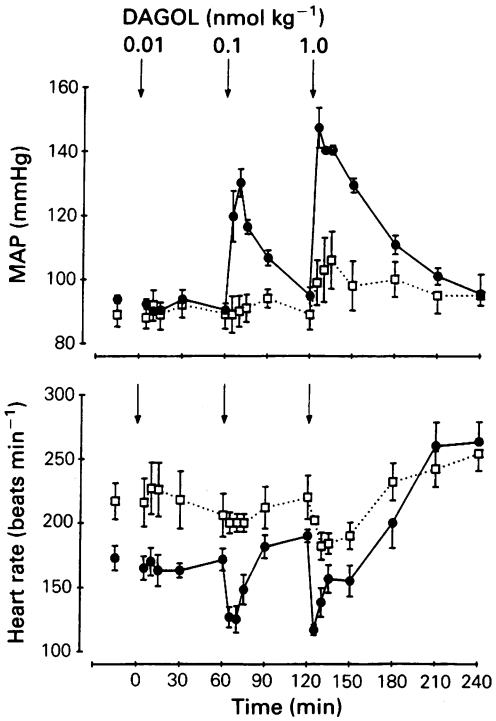

Figure 4 Cardiovascular effects of i.c.v. and i.c. DAGOL in conscious rabbits. After a 30 min control period, three doses of DAGOL (0.01, 0.1 and 1.0 nmol kg^{-1}) were given at 0, 60 and 120 min respectively, either i.c.v. (●) or i.c. (□). The control value is the mean of six readings taken during the control period. Each point represents the mean (i.c.v. $n = 6$, i.c. $n = 5$), with s.e.mean shown by vertical bars.

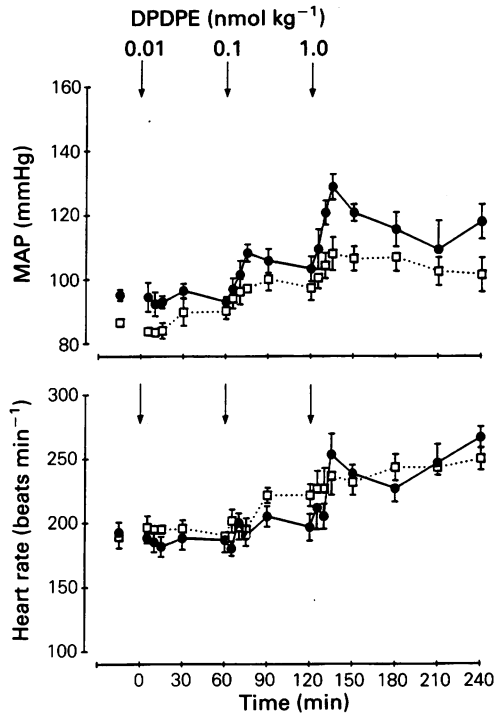

Figure 5 Cardiovascular effects of i.c.v. and i.c. DPDPE in conscious rabbits. After a 30 min control period three doses of DPDPE (0.01, 0.1 and 1.0 nmol kg^{-1}) were given at 0, 60 and 120 min respectively, either i.c.v. (●) or i.c. (□). The control value is the mean of six readings taken during the control period. Each point represents the mean (i.c.v. $n = 6$, i.c. $n = 6$), with s.e.mean shown by vertical bars.

naloxone plasma catecholamines did not fall until 60 min after DAGOL (Table 1). Within 5 min of naloxone the DAGOL-induced respiratory depression was attenuated. Respiration rate increased from 69 ± 8 to 112 ± 29 breaths min^{-1}, $Paco_2$ fell from 44.3 ± 2.5 to 37.8 ± 2.1 mmHg and Pao_2 increased from 72.2 ± 4.7 to 76.5 ± 3.1 mmHg. In the absence of naloxone, respiratory depression increased until 30 min after DAGOL and only began to return to control levels after 90 min (Figure 6). Naloxone also abolished the sedative effect of DAGOL.

DPDPE (1 nmol kg^{-1}) was given i.c.v. followed after 10 min by either i.v. saline (300 μl kg^{-1}) or i.v. naloxone (3 μmol kg^{-1}) ($n = 6$). DPDPE caused a similar increase in MAP and HR to that in the earlier dose-response study. After i.v. naloxone (3 μmol kg^{-1}), MAP and HR returned to control within 10 min; at all times after naloxone MAP was significantly ($P < 0.05$) lower than in the same group of rabbits when saline was given after DPDPE (Figure 7). Naloxone (300 nmol kg^{-1}) injected i.v. had no effect on any of the responses to i.c.v. DPDPE. The effects of naloxone on noradrenaline were unclear as the initial increases after DPDPE were different. Plasma noradrenaline was 0.8 ± 0.2 nmol l^{-1} above control 10 min after DPDPE and 5 min after naloxone (3 μmol kg^{-1}) it was 0.5 ± 0.3 nmol l^{-1} above control. When saline was given after DPDPE the initial rise in noradrenaline was 1.7 ± 0.7 nmol l^{-1} and 5 min after saline it was 1.6 ± 0.4 nmol l^{-1}. The increased activity and alertness caused by DPDPE was abolished by naloxone.

Discussion

The present study demonstrates that in conscious rabbits the μ-receptor agonist DAGOL given i.c.v.

Table 1 Changes in plasma noradrenaline, adrenaline and glucose after i.c.v. and i.c. [D-Ala^2, MePhe^4-Gly^5-ol] enkephalin (DAGOL) in conscious rabbits

			i.c.v.			i.c.		
Treatment	*Dose* (nmol kg^{-1})	*Time* (min)	*Noradrenaline* (nmol l^{-1})	*Adrenaline* (nmol l^{-1})	*Glucose* (mmol l^{-1})	*Noradrenaline* (nmol l^{-1})	*Adrenaline* (nmol l^{-1})	*Glucose* (mmol l^{-1})
Control			1.05 ± 0.14	0.22 ± 0.05	6.36 ± 0.25	1.53 ± 0.33	0.44 ± 0.15	6.78 ± 0.12
DAGOL	0.1	60	1.07 ± 0.11	0.52 ± 0.13	7.03 ± 0.16	1.68 ± 0.59	0.40 ± 0.10	7.02 ± 0.27
DAGOL	1.0	5	4.00* ± 0.29	26.54* ± 2.79		2.48 ± 0.84	2.78 ± 1.19	
DAGOL	1.0	30	4.60* ± 0.73	25.43* ± 5.81	16.66* ± 0.74	2.41 ± 0.77	1.95 ± 0.88	8.53* ± 0.67
DAGOL	1.0	60	3.26* ± 0.25	14.18* ± 1.59	5.34* ± 1.58	2.03 ± 0.48	0.84 ± 0.39	7.81 ± 0.52

After a 30 min control period DAGOL (0.01, 0.1 and 1.0 nmol kg^{-1}) was given at 60 min intervals. Samples were collected at the end of the control period, at 60 min after 0.1 nmol kg^{-1} and at 5, 30 and 60 min after 1.0 nmol kg^{-1}. Values are means ± s.e.mean (i.c.v. $n = 6$, i.c. $n = 5$).
* $P < 0.05$ for comparisons with control.

caused hypertension which was probably due to α-adrenoceptor-mediated vasoconstriction resulting from the high circulating catecholamine levels. A similar mechanism has been shown following intravenous administration of the μ-opioid agonist morphine in conscious rabbits (May *et al.*, 1988; 1989a),

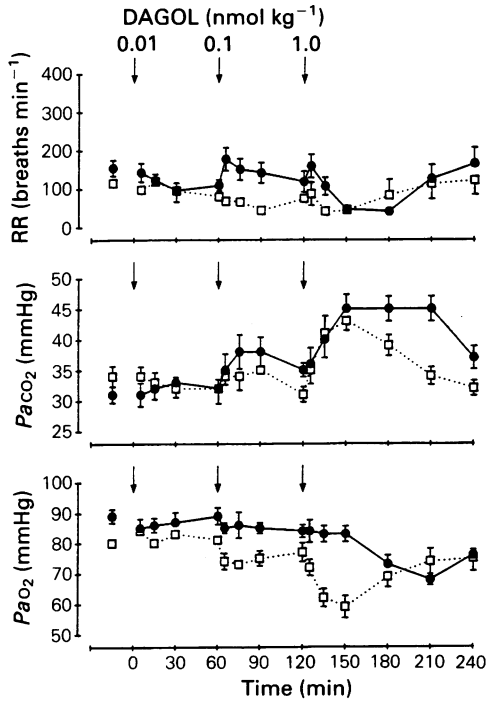

Figure 6 Respiratory effects of i.c.v. and i.c. DAGOL in conscious rabbits. After a 30 min control period three doses of DAGOL (0.01, 0.1 and 1.0 nmol kg^{-1}) were given at 0, 60 and 120 min respectively, either i.c.v. (●) or i.c. (□) (RR = respiration rate). Each point represents the mean (i.c.v. $n = 6$, i.c. $n = 5$), with s.e.mean shown by vertical bars.

and after i.c.v. administration of DAGOL in conscious rats (Pfeiffer *et al.*, 1983). The present findings indicate that the effect of DAGOL is due to an action on periventricular sites as it occurred after i.c.v. but not i.c. administration. Our demonstration of μ-receptors in the hypothalamus, which would be reached rapidly as drugs pass from the lateral into the 3rd ventricle, indicates that this may be a site of action. This is supported by the finding that microinjections of DAGOL into the anterior hypothalamus of conscious rats increased sympatho-adrenal outflow and produced hypertension (Pfeiffer *et al.*, 1982). The accompanying bradycardia probably resulted from a baroreflex-mediated increase in vagal tone. The delayed tachycardia which occurred after the highest dose suggests attenuation of the baroreflex mechanisms at this time, probably by an action on the brainstem as it occurred after i.c. as well as i.c.v. administration. This may result from an action on μ-receptors in the dorsal motor nucleus of the vagus. The hypertension following i.c.v. DAGOL is not secondary to the respiratory depression as there was no increase in BP after i.c. DAGOL when there were similar changes in blood gases to those after i.c.v. DAGOL. This conclusion is supported by the fact that hypertension preceded the onset of respiratory depression.

The δ-receptor agonist DPDPE also caused hypertension but, unlike DAGOL, it was accompanied by a parallel increase in HR. The similar changes after i.c.v. and i.c. administration suggest an action on δ-receptors demonstrated by autoradiography in the NTS rather than on those in the hypothalamus. DPDPE did not induce a generalized increase in sympatho-adrenal outflow but evoked a selective release of noradrenaline with no change in adrenaline. The hypertension following DPDPE may, therefore, result from vasoconstriction in selective vascular beds, and from an increase in cardiac output as the rise in MAP paralleled the rise in HR.

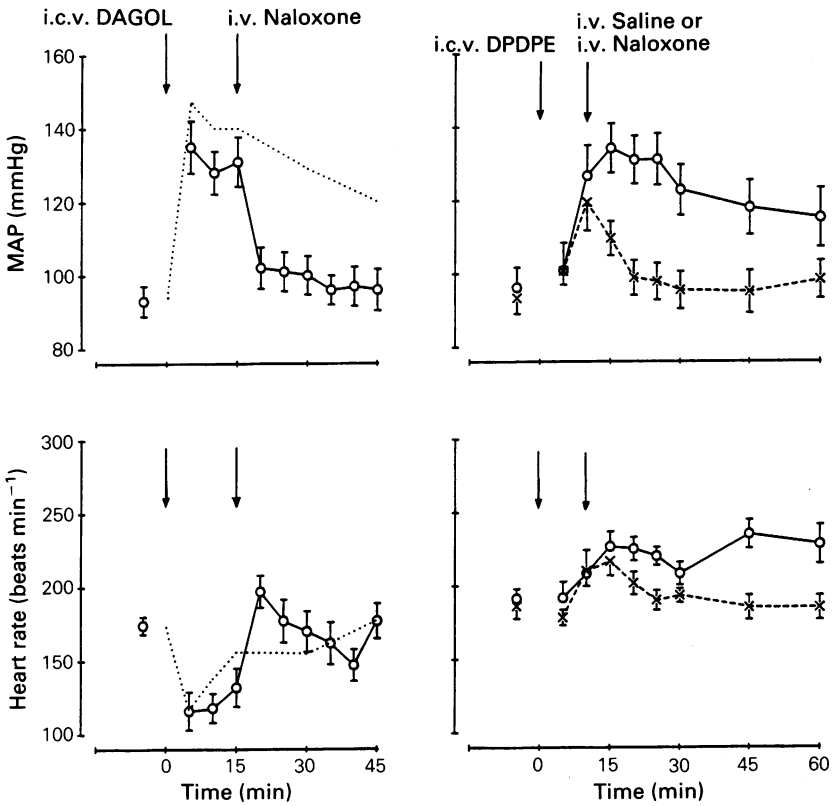

Figure 7 Effect of i.v. naloxone on the cardiovascular responses to i.c.v. DAGOL and DPDPE in conscious rabbits. Left panels: after a 30 min control period, DAGOL (1.0 nmol kg^{-1}) was given i.c.v. at 0 min followed by i.v. naloxone (30 nmol kg^{-1}) at 15 min. For comparison the dotted line represents the changes in MAP and HR seen after i.c.v. DAGOL (1.0 nmol kg^{-1}) taken from Figure 4. Each point represents the mean ($n = 6$) with s.e.mean shown by vertical bars. Right panels: after a 30 min control period DPDPE (1.0 nmol kg^{-1}) was given i.c.v. at 0 min followed either by i.v. naloxone (3 μmol kg^{-1}) (×) or i.v. saline (300 μl kg^{-1}) (○) at 10 min. Each point represents the mean ($n = 6$) with s.e.mean shown by vertical bars.

Table 2 Changes in plasma noradrenaline, adrenaline and glucose after i.c.v. and i.c. [D-Pen^{2,5}]enkephalin (DPDPE) in conscious rabbits

			i.c.v.			i.c.		
Treatment	*Dose* (nmol kg^{-1})	*Time* (min)	*Noradrenaline* (nmol l^{-1})	*Adrenaline* (nmol l^{-1})	*Glucose* (mmol l^{-1})	*Noradrenaline* (nmol l^{-1})	*Adrenaline* (nmol l^{-1})	*Glucose* (mmol l^{-1})
Control			1.11 ± 0.20	0.25 ± 0.06	6.65 ± 0.16	1.52 ± 0.28	0.33 ± 0.17	7.30 ± 0.42
DPDPE	0.1	60	1.33 ± 0.34	0.19 ± 0.04	6.95 ± 0.22	1.47 ± 0.24	0.46 ± 0.36	7.67 ± 0.16
DPDPE	1.0	5	1.56 ± 0.23	0.16 ± 0.05		1.84 ± 0.27	0.27 ± 0.15	
DPDPE	1.0	30	2.00 ± 0.53	0.23 ± 0.04	7.56* ± 0.29	1.70 ± 0.14	0.40 ± 0.29	7.87 ± 0.25
DPDPE	1.0	60	1.32 ± 0.11	0.38 ± 0.12	7.81* ± 0.29	2.34* ± 0.14	0.66 ± 0.52	8.53 ± 0.31

After a 30 min control period DPDPE (0.01, 0.1 and 1.0 nmol kg^{-1}) was given at 60 min intervals. Samples were collected at the end of the control period, at 60 min after 0.1 nmol kg^{-1} and at 5, 30 and 60 min after 1.0 nmol kg^{-1}. Values are means ± s.e.mean (i.c.v. and i.c. $n = 6$).

* $P < 0.05$ for comparisons with control.

The changes in MAP and HR after DPDPE are similar to those caused by i.c.v. and i.c. administration of leucine-enkephalin in conscious rats (Schaz *et al.*, 1980) suggesting that these effects of leucine-enkephalin resulted from stimulation of δ-opioid receptors. Comparison with other studies is difficult as [D-Ala^2, D-Leu^5]enkephalin has frequently been used as a δ-selective ligand but, as stated by the authors, it also has significant effects at μ-receptors (Pfeiffer *et al.*, 1982).

It is unlikely that the different responses to DAGOL and DPDPE result from different tissue distribution of the agonists in the brain leading to stimulation of separate populations of μ-receptors for two reasons. Firstly both agonists are highly specific particularly at the relatively low doses used. Secondly, the effects of DAGOL were inhibited by low doses of the μ-selective antagonist naloxone, whereas much higher doses were required to antagonize the effects of DPDPE, consistent with an action on δ-receptors.

The κ-receptor agonists U69593 and dynorphin had no effects on MAP but at the highest doses they caused a small elevation of HR. In contrast, a fall in HR has been shown in anesthetized rats after microinjection of dynorphin into the NTS and nucleus ambiguus (Hassen *et al.*, 1984), and microinjection of the selective κ-agonist U50488 into the dorsal motor nucleus of the vagus also reduced HR (Hassen, 1985). These differences from the present findings may result from the higher doses used, the use of anaesthetics or the different sites of administration. The similar effects after dynorphin and U69593 in the rabbit indicate that U69593 acts on the same population of κ-receptors as this endogenous κ-agonist. These findings correlate with the autoradiographic studies which were unable to demonstrate κ-binding using U69593 in rabbit hypothalamus or brainstem. The low degree of binding demonstrated in the hypothalamus using EKC may be a result of binding to μ- or δ-receptors, since only low concentrations of unlabelled DAGOL and DPDPE were included with the [^{3}H]-EKC incubation solutions.

These studies have also enabled investigation of the receptor subtype involved in opiate-induced respiratory depression which is of importance as it is a major side effect of narcotic analgesics. The respiratory depressant effects of morphine depend on stimulation of μ_2-receptors (Ling *et al.*, 1983), but a role for δ-receptors in opiate-induced respiratory depression has also been suggested (Pazos & Florez, 1984) and κ-agonists have been shown to reduce respiratory rate and tidal volume (Hassen *et al.*, 1984). Our results, using more specific agonists in conscious animals, suggest that opioid-induced respiratory depression is mediated entirely by μ-receptors. There is no evidence indicating a role for δ- or κ-receptors as DPDPE had no effect on respiration rate or blood gases and the κ-agonists reduced $PaCO_2$ after i.c.v. administration. This is also supported by the finding that the respiratory effects of DAGOL are blocked by low doses of naloxone which would not be expected to inhibit effects at δ- or κ-receptors. The respiratory depression occurred after i.c.v. and i.c. administration indicating that the receptor sites involved are probably in the brainstem.

These studies have demonstrated striking differences in the localisation of opioid receptor subtypes in hypothalamic and brainstem nuclei associated with cardiovascular control. Taken together with the specific functional effects of the agonists these findings are further evidence of role for opioid peptides in the central regulation of cardiovascular function. The hypertensive action of DAGOL and DPDPE indicates that the endogenous μ- and δ-ligands may be involved in the pathogenesis of hypertension. The ability of naloxone to reduce BP in spontaneously hypertensive rats (Wexler, 1984) and to prevent the increase in BP following renal artery constriction (Szilagyi *et al.*, 1986) supports a role for opioid peptides in hypertension. Naloxone also attenuates the hypertension in response to stress (Naranjo *et al.*, 1986) indicating that opioids may mediate stress-induced cardiovascular changes. In contrast, the ability of naloxone to reduce the hypotension following various forms of shock (Holaday, 1983) indicates that in this situation opioid peptides are acting to reduce BP. Thus opioid peptides have multiple effects on the cardiovascular system depending on the receptor subtype stimulated, the site of administration and the state of the animal. Further studies are required to determine the physiological role of endogenous opioid peptides in cardiovascular control and their possible involvement in the cardiovascular responses to stress.

C.N.M., C.J.W. and C.J.M. (Wellcome Senior Lecturer) are grateful for support from the Wellcome Trust. M.R.D. is grateful for support from the British Heart Foundation.

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(Received January 5, 1989
Revised April 4, 1989
Accepted July 4, 1989)