Opiate Analogs, Substance P, and Baroreceptor Reflexes in the Rabbit

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SUMMARY The putative transmitters, enkephalins and substance P, and their binding sites have been identified in the nucleus of the solitary tract. Their role in the modulation of baroreceptor reflex activity is the subject of this study in the rabbit. A stable decarboxy analog of leu-enkephalin, RX783016, which has \( \mu \) receptor specificity, was used to attenuate the baroreflex sensitivity to intravenous phenylephrine. RX783016, 50 \( \mu \)g/kg intracisternally, did not alter resting heart rate or blood pressure. Intravenous administration of the opiate receptor antagonist, naloxone, prevented the effects of RX783016. Naloxone given alone significantly increased reflex sensitivity. Substance P given intracisternally in low doses (1 to 10 ng/kg) caused a dose-dependent pressor response, which was reduced by pretreatment with morphine and enhanced by naloxone. Bilateral sinoaortic denervation also enhanced the pressor response to substance P, but after deafferentation, naloxone had no further effect. It is proposed that enkephalin-containing neurons, acting through \( \mu \) receptors, and substance P neurons influence baroreceptor reflex activity by modulating respectively the primary and second order neurons of the baroreceptor reflex. (Hypertension 3 (supp I): 1-142-1-147, 1981)

KEY WORDS • blood pressure • baroreceptor reflexes • substance P enkephalins • naloxone • central nervous system

THE role of monoamines in central cardiovascular regulation, in general, and the central connections of the baroreceptor reflex pathway, in particular, have been extensively studied. There is evidence that central norepinephrine and serotonin may contribute as transmitters or modulators in efferent sympathetic pathways. Many other transmitters and modulators have been proposed to contribute to baroreflex and chemoreceptor reflex mechanisms. These include central angiotensin II (AII) and other peptides including enkephalins and substance P.

Substance P, a putative sensory transmitter, is found in large amounts in the nucleus of the solitary tract, the site of the primary synapse of many afferent baroreceptor fibers. Substance P may be the sensory transmitter of the baroreceptor reflex. The pentapeptide endogenous opioids or enkephalins are also found in large amounts in the nucleus of the solitary tract as are specific binding sites or opiate receptors. It has been proposed that enkephalins released from interneurons in the dorsal horn of the spinal cord act to modify sensory input. The distribution of enkephalins in the brain stem is consistent with such a neuromodulator role influencing the expression of afferent baroreceptor input.

Recently, it has been reported that several types of opiate receptor can be distinguished in isolated peripheral tissues and also in the central nervous system. This classification into \( \mu \), \( \delta \), and \( \kappa \) receptor types is based on the relative activity of a range of agonists and antagonists, and these agents can be used to determine the relative contributions of these receptor types to responses studied.

In the present study, we examined in the rabbit the effect of naloxone, an antagonist of \( \mu \) receptors, and several exogenous opiate agonists with \( \mu \), \( \delta \), and \( \kappa \) activity, on baroreceptor reflex function. The actions of centrally administered substance P on blood pressure, heart rate, and baroreflex activity was also studied.

Methods

Blood Pressure Recording

All studies were performed on male New Zealand white rabbits (Hyline Ltd., Cheshire, England), weighing 2.2 to 2.7 kg. In studies performed on conscious rabbits, a polypropylene arterial catheter was introduced into the central artery of the ear under local anesthesia (1% lignocaine) at least 60 minutes before the study. For intravenous drug administration, a further catheter was inserted into the central vein of the ear and advanced 6 to 8 cm toward the heart and...
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great veins. The catheters were taped to the ear, and the animals were maintained unrestrained in individual wooden cages, 20 × 20 × 50 cm, for the duration of the experiment. In studies performed under light intravenous sodium pentobarbitone anesthesia, blood pressure was either measured as described above or from a catheter introduced into the femoral artery. Arterial pressure was recorded using a strain gauge transducer and displayed on a Grass Polygraph. Mean arterial pressure (MAP) was determined electronically from the phasic trace. Heart rate was measured from the arterial pressure recording.

Sinoaortic Denervation

Bilateral baroreceptor deafferentation was performed 7 to 14 days before study. Under pentobarbitone anesthesia, the aortic depressor nerve was sectioned bilaterally after identification in the carotid and neurovascular bundle.2 The carotid sinus was denervated on both sides by stripping nerve fibers and adventitia from the sinus and the common, internal and external carotid artery for at least 2 cm from the bifurcation. To accomplish complete deafferentation, the sinus and bifurcation were painted with 10% phenol. The completeness of baroreceptor denervation was assessed by the absence of bradycardia after intravenous injections of norepinephrine and All in doses that raised the MAP by 50 to 70 mm Hg.

Baroreceptor Reflex Sensitivity

Sensitivity of the baroreceptor reflex was assessed by a modification of the method of Smyth et al.14 The heart rate response to increases in blood pressure induced by intravenous phenylephrine (10 μg/kg) was examined 15 to 30 minutes after vehicle injection i.c. and after administration of antagonist drugs, peptides, or other agonists. The blood pressure and heart rate were recorded at a fast chart speed to allow measurement of individual intervals between systolic pressure waves (heart period or the reciprocal of heart rate) which was plotted against the preceding MAP. At least 10 separate heart periods and MAP data points were used to derive a MAP:heart period relationship. There was a highly significant linear relationship (r values ranged from 0.84 to 0.98) in all cases. The slope of this relationship was used as an index of sensitivity of the baroreflex sensitivity.15

Drugs and Administration Protocol

Intravenous (i.v.) drug administration was performed in a total volume not exceeding 2 ml. Intracisternal (i.c.) injection was performed free-hand transcutaneously using a 27-gauge flat bevel needle and a Hamilton microtiter syringe. The total volume injected did not exceed 0.1 ml. As previously described,2 the i.c. injection was performed under light pentobarbitone anesthesia. With the animal resting prone, the neck was flexed and the needle introduced transcutaneously through the atlanto-occipital membrane.

Phenylephrine was obtained from Sigma Chemical Company, London, dissolved in 0.9% saline. RX783016 (Tyr-D-ala Gly-Me NH (CH3)3N ME2) (a μ agonist)17 and ketocyclazocine (a proposed κ agonist)18 were gifts from Reckitt and Colman Ltd., Hull, England; and BW 180C (Tyr-D-ala-Gly-Phe-D-leu) (a δ agonist)19 was obtained from Wellcome Laboratories, Beckenham, England. Naloxone was purchased from Endo Laboratories, England, and used from commercially available ampules, as was morphine. Substance P was purchased from Uniscience, Cambridge, England. Substance P, BW 180C, and RX783016 were dissolved in 0.9% saline and ketocyclazocine in acetic acid diluted to 0.125 μM. Responses to all drugs were compared to control experiments where the appropriate vehicle was administered.

Naloxone was given i.v. in both conscious and anesthetized rabbits, and baroreflex function was assessed 15 minutes later. Opiate agonists (RX 783016, BW 180C, and ketocyclazocine) were injected i.c. under light pentobarbitone anesthesia, and the effect on baroreflex sensitivity determined after 30 minutes. To study the effect of naloxone on opiate responses, naloxone was given i.v. 15 minutes after the i.c. administration of the opiate, and the reflex function was tested 15 minutes after naloxone (and, thus, 30 minutes after the opiate).

Substance P was given by i.c. injection, and blood pressure and heart rate was measured at intervals for 60 minutes. A further group of rabbits were pre-treated with i.v. naloxone or morphine 15 minutes before Substance P was given i.c.

Substance P, BW 180C, and RX783016 were dissolved in 0.9% saline and ketocyclazocine in acetic acid diluted to 0.125 μM. Responses to all drugs were compared to control experiments in which the appropriate vehicle was administered i.v. or i.c.

Data Analysis

In all experiments, groups of 10 rabbits were studied and results expressed as mean ± sp. Responses in groups of animals treated with the appropriate vehicle only were compared with active treatments. Where appropriate, differences between means were compared using either parametric (Student's "t" test) or nonparametric (Whitney Mann "U" test) tests. The differences between slopes of the regression in relationships of MAP and heart period were compared by two-way analysis of variance and application of the Scheffé test.

Results

Opiate Agonists and Baroreflex Sensitivity

Baroreflex sensitivity determined as the slope of the pressure-heart period relationship following intravenous phenylephrine was compared after opiate agonists with varying selectivity for opiate receptor types in anesthetized rabbits.
RX783016, 50 μg/kg, intracisternally significantly reduced baroreflex sensitivity \( (p < 0.01) \) from 2.2 ± 0.6 to 1.3 ± 0.7 msec/mm Hg when compared to saline vehicle injection in anesthetized rabbits (fig. 1). After preliminary experiments, the dose of agonist was selected as that dose which caused consistent changes in baroreflex activity without itself significantly influencing baseline blood pressure and heart rate. The effects of ketocyclazocine and BW 180C are also shown in figure 1. Ketocyclazocine and BW 180C increased baroreflex sensitivity in response to phenylephrine (fig. 1). There was no evidence of respiratory depression after the doses of opiate agonists used, as arterial carbon dioxide tension (Corning 175 analyses) was not significantly changed.

**Opiate Receptor Antagonism with Naloxone and Baroreflex Sensitivity**

Pentobarbitone anesthesia reduced baroreflex sensitivity (fig. 2). Naloxone (80 μg/kg) given intravenously, which had no effect on baseline MAP or heart rate, increased the slope of the pressure-heart period relationship and thus increased baroreflex sensitivity when given alone to conscious or anesthetized rabbits (fig. 2). Although this intravenous dose of naloxone did not reverse the effects of RX783016, a higher dose (200 μg/kg) attenuated significantly \( (p < 0.01) \) the inhibitory effect of the opioid peptide on baroreflex sensitivity (fig. 1).

Naloxone (200 μg/kg) alone did not change reflex sensitivity significantly but at this dose level it also attenuated the effect of BW 180C, a δ agonist (fig. 1), which had the opposite effect on the reflex.

**Central Effects of Substance P in the Rabbit**

In preliminary experiments, the effect of intravenous and intracisternal substance P were compared. Intravenously a fall in blood pressure was observed which contrasted with the pressor response observed with low doses given intracisternally (fig. 3). Central injection of substance P resulted in bradycardia.

When a range of intracisternal doses was examined, the dose-response relationship was not linear; it appeared that at higher doses \( (> 20\ ng) \) the central pressor response was not only reversed but a hypotensive action was also seen. However, hypotension was not only seen at doses that lowered blood pressure when given intravenously, but at other times as well, and could represent peripheral leakage of the central injection. The pressor response was short-lasting (15 minutes) and was observed after central administration of small doses (5 ng/kg), which had no measurable hemodynamic effect when given intravenously.

**Substance P, Opiates, and Baroreflex Function**

Pretreatment of anesthetized rabbits with naloxone, 200 μg/kg i.v., 15 minutes before substance P was given, significantly enhanced the pressor response to intracisternal substance P \( (< 0.05) \) and reduced the bradycardia \( (p < 0.01) \). However, morphine 1 mg/kg intravenously attenuated the pressor response to central substance P (fig. 4).

Effects of intracisternal substance P were modified by surgical denervation of the arterial baroreceptors by bilateral sinoaortic denervation done 7 to 14 days.
previously. The pressor response to 5 ng/kg was significantly increased (fig. 5) in denervated animals and the bradycardia abolished. In denervated rabbits, however, naloxone pretreatment did not further enhance the response to substance P (fig. 5).

Discussion

These studies provide further evidence implicating opiate receptor mechanisms in the modulation of baroreceptor reflex activity. Previous workers have examined the effects of peptide analogs of enkephalins and nonpeptide opiate agonists, and concluded that opiate receptors facilitate or attenuate reflex activity. By using opiate agonists with differing specificity for the proposed μ, δ and κ types of receptors, it is apparent that opiate agonists may have different effects depending on the agonist specificity. Opiate receptors of the μ type, which are stimulated by the descarboxy analog of leu-enkephalin (RX

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**Figure 3.** The effect of substance P (5 ng/kg) intracisternally (open circles) and intravenously (black circles) on mean arterial pressure and heart rate of pentobarbitone-anesthetized rabbits \( n = 8, \) mean ± sd.

**Figure 4.** The effect of morphine \( (1 \text{ mg/kg}) \) intravenously and naloxone \( (200 \mu \text{g/kg}) \) intravenously on the pressor response to intracisternal substance P \( (5 \text{ ng/kg}) \) in pentobarbitone anesthetized rabbits \( n = 8, \) mean ± sd; \* \( p < 0.05 \).

**Figure 5.** The effect of bilateral sinoaortic denervation 7 to 14 days before and naloxone \( (200 \mu \text{g/kg}) \) intravenously on the pressor response to intracisternal substance P \( (5 \text{ ng/kg}) \) in pentobarbitone-anesthetized rabbits \( n = 8, \) mean ± sd; \* \( p < 0.05 \), \* \( p < 0.05 \) when control substance P responses are compared to other treatments by repeated measures (two-way analysis of variance and Scheffe test).
appear to attenuate baroreflex sensitivity. Naloxone, which is a relatively specific µ antagonist, blocks the effect of RX783016. Intravenous naloxone alone increased the sensitivity of baroreflex responses when tested with phenylephrine. These effects of naloxone support the hypothesis that endogenous opiates of the µ type participate in baroreflex regulation in the normal rabbit. It is particularly important to note that neither naloxone nor the opiate agonists in the doses used in this study had significantly changed arterial pressure or heart rate at the time of the reflex study. Changes in baseline heart rate and pressure can make interpretation of baroreflex sensitivity changes difficult. Agonists with relative specificity for δ and κ receptor respectively, BW 180C and ketocyclazocine increased baroreflex sensitivity, suggesting that these receptor types may exert opposite effects to µ receptors on baroreceptor reflex function.

Although our results are consistent with a recent report that intracisternal enkephalins attenuated baroreflex responses in cats and rats, and that these effects were blocked by naloxone, 20 they offer an explanation for contradictory reports in the literature 21, 22 based on a heterogeneity of opiate receptor types and responses. Substance P injected into the cisterna magna raised blood pressure and lowered heart rate. In previous experiments, we have observed that doses higher than 10 to 20 ng/kg may be associated with hypotension of a magnitude similar to that after intravenous injection. A pressor response to intracisternal substance P has been observed by others 21, 22 although heart rate changes are more variable. Recently it has been reported that, in cats and rats, substance P by microinjection (5 to 15 nmole) or direct application to the obex (4 nmole) lowers blood pressure and heart rate under urethane anesthesia. 2 The doses used to demonstrate this fall in blood pressure are orders of magnitude greater than those used in the present study (6.75 pmole/kg) or in other reports. 21, 22

The central pressor response to substance P is mediated by an increase in efferent sympathetic outflow as it is blocked by intravenous pentolinium and attenuated by peripheral aδ adrenergic blockade. The bradycardia is mediated, at least in part, via secondary activation of baroreceptor reflex mechanisms. The opiate receptor antagonist, naloxone, increases the pressor response to substance P while the µ receptor agonist, morphine, attenuates it. Enhancement of the pressor response to substance P by intracisternal naloxone has been described in the α chloralose anesthetized rat. 21 Bilateral sinoaortic denervation increases the pressor responses to substance P and prevents the bradycardia.

While this may not be unexpected, the failure of naloxone to further modify the response to substance P in denervated animals suggests that endogenous opiates unlike substance P require the integrity of baroreflex afferents to modify reflex function.

Thus, both opiates and substance P modulate baroreflex activity at the level of the brain stem, and the actions of the two are closely related. However, as the peptides were administered intracisternally in the present study, it is difficult to precisely localize the sites of action in the brain stem. The results presented justify further experiments with more localized drug application to the nucleus of the solitary tract and adjacent areas. It is possible to speculate on the basis of the present results and to examine several putative models of the interaction of these peptide systems and the primary and second order neurons of the baroreflex arc. Although it has been proposed that substance P might itself be the transmitter at the first synapse of the baroreflex, 7, 21, 22 and that enkephalin interneurons may modulate presynaptically the release of substance P, this explanation is not supported by the enhancement of the effect of exogenous substance P by naloxone or the failure of naloxone to enhance substance P after sinoaortic denervation.

Likewise, several other possible models are not consistent with the results presented, including both substance P and enkaphalin modulating the second order neuron or either peptide participating as transmitter for a second order neuron. It could be speculated that an enkephalin containing neuron via µ receptor modulates presynaptically the release of the sensory transmitter from the baroreceptor afferents. Substance P modifies the responses of the second order neuron. The pressor response observed after central substance P in the present study presumably results from facilitation of the sympathetic outflow or partial disinhibition of the baroreflex input.

In conclusion, both enkephalins and substance P appear to participate in cardiovascular regulation at the level of the brain stem and, in particular, play a role in the modulation of baroreceptor reflex activity.

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