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NALOXONE REVERSES THE ANTIHYPERTENSIVE EFFECT OF CLONIDINE

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In unanaesthetized, spontaneously hypertensive rats the decrease in blood pressure and heart rate produced by intravenous clonidine, 5 to 20 μg/kg, was inhibited or reversed by naloxone, 0.2 to 2 mg/kg. The hypotensive effect of 100 mg/kg α-methyldopa was also partially reversed by naloxone. Naloxone alone did not affect either blood pressure or heart rate. In brain membranes from spontaneously hypertensive rats clonidine, 10^{-8} to 10^{-5} M, did not influence stereoselective binding of [^{3}H]-naloxone (8 nM), and naloxone, 10^{-8} to 10^{-4} M, did not influence clonidine-suppressible binding of [^{3}H]-dihydroergocryptine (1 nM). These findings indicate that in spontaneously hypertensive rats the effects of central α-adrenoceptor stimulation involve activation of opiate receptors. As naloxone and clonidine do not appear to interact with the same receptor site, the observed functional antagonism suggests the release of an endogenous opiate by clonidine or α-methyldopa and the possible role of the opiate in the central control of sympathetic tone.

Introduction Clonidine is a potent antihypertensive agent that reduces blood pressure by decreasing sympathetic tone (Schmitt, Schmitt, Boissier & Giudicelli, 1967). Activation of α-adrenoceptors in the medulla oblongata is believed to mediate this effect (Kobinger & Walland, 1971; Schmitt, Schmitt & Fenard, 1971), although histamine H_2-receptors (Karppanen, Paakkari, Paakkari, Huotari & Orma, 1976; Finch, Harvey, Hicks & Owen, 1978) and muscarinic cholinoceptors (Srimal, Gulati & Dhawan, 1977) have also been implicated. Morphine-like compounds acting on opiate receptors in the medulla can also decrease sympathetic tone and arterial blood pressure (Laubie, Schmitt, Canellas, Roquebert & Demichel, 1974; Feldberg & Wei, 1977). Clonidine, on the other hand, was shown to have antinociceptive effects (Fielding, Wilker, Hynes, Szewczak, Novick & Lal, 1978) and to reverse opiate withdrawal symptoms (Gold, Redmond & Kleber, 1978). Also, the symptoms of withdrawal of opiates (Gold *et al.*, 1978) and clonidine (Geyskes, Boer & Durhout Mees, 1978) are remarkably similar. Although clonidine and opiates act on distinct receptors in the central nervous system (Aghajanian, 1978), the mutual overlap in their effects suggests significant overlap in the effector pathways of the central adrenergic and opiate receptor systems. Some effects of opiates, as well as symptoms of their withdrawal, may be mediated by altered activity of noradrenergic neurones in certain brain areas (Gold *et al.*, 1978; Aghajanian, 1978). By analogy, it is possible that some effects of clonidine may involve direct or indirect activation of opiate receptors. We describe here how the opiate antagonist, naloxone, inhibits the decrease in blood pressure and heart rate produced by clonidine in spontaneously hypertensive rats; these effects appear not to be due to an interaction of the two drugs with the same receptor site.

Methods Experiments were done in male, 300 to 350 g, spontaneously hypertensive rats of the Okamoto-Aoki strain. Systolic blood pressure and heart rate of unanaesthetized animals were measured by the tail cuff technique. The rats were placed in a restrainer maintained at 37°C and were allowed to settle down over a period of 30 min. Pulsations of the tail artery, detected by a BioCom piezo-electric microphone, were monitored by a sphygmomanometer pre-amplifier (Grass 7P8E) and a polygraph. Baseline blood pressure and heart rate values were obtained as a mean of 10 to 12 measurements. The effects of various drugs on these parameters were assessed from measurements (average of 3 tests) obtained at 5 min intervals for periods up to 4 h following drug administration. Clonidine hydrochloride and sodium nitrite were given into a tail vein, whereas α-methyldopa and naloxone hydrochloride were administered intraperitoneally. Injection of 0.9% w/v NaCl solution (saline) in a volume and through a route identical to those used for drugs, did not significantly alter blood pressure or heart rate.

For receptor binding studies, membranes from whole brain of hypertensive rats were prepared and opiate receptor binding assayed according to Pert & Snyder (1973). Receptor-specific binding of [^{3}H]-naloxone (8 nM) was defined as the difference in binding in the presence of 1 μM levorphanol and 1 μM

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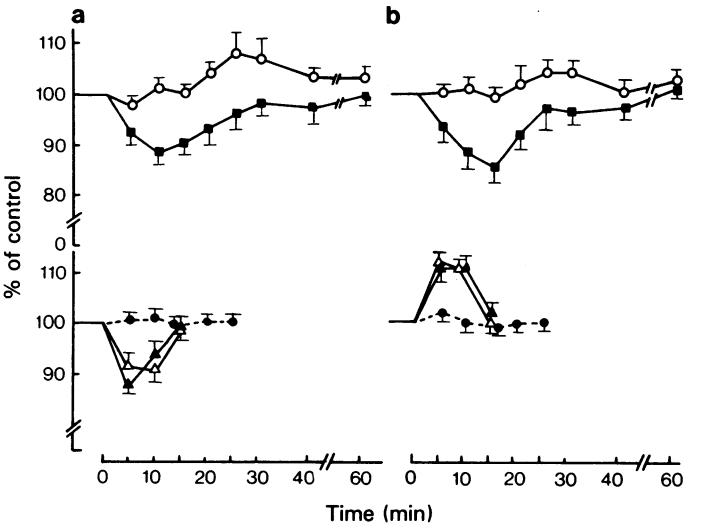

Figure 1 The effect of naloxone on blood pressure (a) and heart rate (b) responses to clonidine and sodium nitrite. Clonidine HCl, 5 μg/kg, (upper panels), or sodium nitrite, 10 mg/kg, (lower panels), was injected into a tail vein at 0 min in the absence of naloxone (filled symbols) or 5 min after the intraperitoneal injection of 2 mg/kg naloxone HCl (open symbols). In the lower panels (●---●) shows the effects of 2 mg/kg naloxone alone on blood pressure and heart rate. The results are means of measurements in 6 spontaneously hypertensive rats of the Okamoto-Aoki strain; vertical bars show s.e. mean. The mean blood pressure of the rats before and after naloxone was 192.0 ± 1.3 and 194.8 ± 1.7 mmHg, and the mean heart rates 424.0 ± 2.8 and 431.4 ± 6.3 beats/min respectively (100% values).

dextrorphan, and it was 209 ± 28 fmol/mg protein. α-Receptor binding sites in the same membrane preparations were identified by [^{3}H]-dihydroergocryptine (1 nM), as described by Greenberg, U'Prichard, Sheehan & Snyder (1978). Specific binding was defined as binding suppressible by 10^{-5} M clonidine, and it was 205 ± 47 fmol/mg protein or 62 ± 8% of total binding. Binding in the absence and presence of competing ligands was determined in triplicate samples from at least 4 different animals.

Results Figure 1 shows the effect of naloxone (2 mg/kg) on blood pressure and heart rate responses induced by clonidine (5 μg/kg) or by sodium nitrite (10 mg/kg). Clonidine rapidly reduced systolic blood pressure (a) and heart rate (b), and both effects lasted approximately 30 min. Naloxone, given 5 min before clonidine, completely eliminated both responses and unmasked a small rise in blood pressure after clonidine. On the other hand, the decrease in blood pressure and the reflex tachycardia produced by sodium nitrite were not affected by naloxone. As illustrated by the dashed line, naloxone itself did not influence either blood pressure or heart rate. The effects of both clonidine and naloxone were dose-dependent. Clonidine dose–response curves (5, 10 and 20 μg/kg) were shifted to the right by naloxone, and the lowest dose that produced significant inhibition was 0.2 mg/kg. The inhibitory effect of naloxone was also evident when it was administered after clonidine; when 2 mg/kg naloxone was given 10 min after the administration of 5 μg/kg clonidine, both blood pressure and heart rate returned to control levels within 5 min.

In other experiments we found a similar antagonism by naloxone of the hypotensive action of α-methyldopa. α-Methyldopa, 100 mg/kg, was given intraperitoneally to 4 hypertensive rats. Systolic blood pressure gradually declined over a period of 90 min from 201 ± 1 to 139 ± 7 mmHg and remained at this low level for at least 2 h. Intraperitoneal injection of saline during this period did not influence blood pressure, whereas the injection of 2 mg/kg naloxone produced a rapid rise in pressure to 168 ± 8 mmHg, followed by a gradual decline over a 60 min period back to the preinjection level.

To test whether or not the antagonism by naloxone of the antihypertensive effect of clonidine involves a direct interaction of the two drugs at the same receptor, we measured their effects on ligand binding to

opiate and α-receptor sites in a crude membrane preparation from the brain of spontaneously hypertensive rats. Clonidine in concentrations of 10^{-8} to 10^{-5} M did not influence [^{3}H]-naloxone binding in the presence of dextrorphan; only at 10^{-4} M did clonidine slightly suppress ($22 \pm 11\%$) specific binding of [^{3}H]-naloxone. This makes the possibility that clonidine interacts with opiate receptors *in vivo* unlikely, as the highest dose of clonidine used (20 μg/kg) can be calculated to yield a peak blood concentration of less than 10^{-7} M (Jarrott & Spector, 1978). A possible interaction of naloxone with α-receptor binding sites was also tested. At the low concentration used, [^{3}H]-dihydroergocryptine ([^{3}H]-DHEC) appears to label functional α-receptors (Kunos, Hoffman, Kwok, Kan & Mucci, 1979). Whereas clonidine was highly potent ($IC_{50} < 10^{-8}$ M) in suppressing [^{3}H]-DHEC binding, no suppression occurred with naloxone up to 10^{-4} M. As the concentration of naloxone in the cerebrospinal fluid after an intravenous injection of 2 mg/kg was found to be between 10^{-7} and 10^{-6} M (Fu, Halenda, Lawrence, Chau-Pham, Martin & Dewey, 1979), it is unlikely that naloxone inhibited the hypotensive effect of clonidine by blocking central α-adrenoceptors.

Discussion The results presented indicate a specific interaction between central α-adrenoceptor stimulation and an antagonist of opiate receptors in spontaneously hypertensive rats. The lack of competition between clonidine and naloxone at opiate or α-receptor binding sites makes it unlikely that the observed functional antagonism is due to a direct interaction of the two compounds at the same receptor. It also confirms functional evidence for distinct receptors for clonidine and opiates in the central nervous system (Aghajanian, 1978). The possibility of a direct interaction at histamine H_2 or muscarinic receptor sites (see Introduction) is discounted by our finding that naloxone also inhibited the hypotensive action of α-methyldopa, which does not involve H_2-receptors (Finch *et al.*, 1978) and is not known to involve cholinoceptors either. Alternatively, the effects of central α-adrenoceptor stimulation may involve the release of an endogenous opiate and naloxone may block the release or the effect of such a substance. Although the present findings do not provide direct evidence for such a mechanism, they are compatible with it. β-Endorphin and some enkephalins were shown to produce hypotension and bradycardia in dogs (Laubie, Schmitt, Vincent & Remond, 1977), and relatively high doses of naloxone, comparable to those used in the present experiments, were required to antagonize these effects transiently. Naloxone may also act by inhibiting the release of an endogenous opiate; it was recently reported that a morphine-induced increase in opiate-like activity in the cerebrospinal fluid of rabbits can be reduced by 2 mg/kg naloxone (Fu *et al.*, 1979).

Naloxone was recently shown to antagonize the hypotensive effect of endotoxin (Holaday & Faden, 1978) or inhalational anaesthetics (Arndt & Freye, 1979). Such observations and the results presented here raise the possibility of an 'opioidergic' component in the neural pathways controlling sympathetic tone.

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