toxan in rodents and in man; half of the Cytoxan is eliminated within hours after cessation of long-term systemic treatment (*13*). These data suggest that behavioral testing of the F_1 generation might be used as an end point to detect abnormalities induced during spermatogenesis and transmitted through the sperm. This end point would then be used to evaluate genetically transmissible effects of potentially mutagenic, carcinogenic, or teratogenic compounds.

Further studies will determine if the observed differences are transient or permanent in nature. In either case, however, the procedures used in this report indicate a transmissible effect which can be used to detect genetically toxic compounds.

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References and Notes

1. R. W. Kapp, presentation at workshop on the Assessment of Reproductive Hazards in the Workplace, Washington, D.C., 1978.
2. A. J. Wyrobek and W. R. Bruce, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4425 (1975).
3. N. L. A. Cacheiro and W. L. Russell, paper presented at the Environmental Mutagenesis Society, Nashville, Tenn., March 1980, program abstract Cb-13.
4. A. J. Wyrobek and W. R. Bruce, in *Chemical Mutagens, Principles and Methods for Their Detection*, A. Hollaender and F. J. de Serres, Eds. (Plenum, New York, 1978), vol. 5.
5. K. Brady, Y. Herrera, H. Zenick, *Pharmacol. Biochem. Behav.* **3**, 561 (1975).
6. The male and female rats were derived from stock from the National Institute of Mental Health and were in the seventh generation.
7. Modified dominant lethal protocol as described by S. Green *et al.* [*Toxicol. Appl. Pharmacol.* **39**, 549 (1977)].
8. The number of pups in the litters of the CP ×CP crosses were 7, 4, and 1; of the CP × saline crosses, 10, 8, 7, 4, 4, 3, 2, 2, and 1; and from the saline × saline crosses, 10, 10, 10, 9, 9, 8, 8, 8, 6, and 4. These data suggest a certain degree of embryotoxicity as seen by decreased litter size in the CP-treated groups (the mean is 4.0 neonates per litter in CP × CP crosses, 4.6 in CP × saline crosses, and 8.2 in saline × saline crosses). The CP-treated females also had decreased numbers of successful matings (CP × CP, 3 out of 10 and saline × CP, 0 out of 10 compared to CP × saline, 9 out of 10 and saline × saline, 10 out of 10).
9. R. L. Brunner, C. V. Vorhees, L. Kinney, R. E. Butcher, *Neurobehav. Toxicol.* **1**, 79 (1979); C. V. Vorhees, R. E. Butcher, R. L. Brunner, T. J. Sobotka, *Toxicol. Appl. Pharmacol.* **50**, 267 (1979); R. E. Butcher, E. Wootten, C. V. Vorhees, *Mutagen. Carcinog. Teratogen.* **1**, 49 (1980).
10. Surface righting was tested daily beginning on day 3. The pups were given two daily trials with a maximum of 30 seconds per trial. The time required for the pup to right itself so that all four feet were on the surface was recorded. The criterion was that the pup had to right itself on at least one single trial in less than 2 seconds on a given day.
11. A statistical analysis of the percentage of pups in each litter reaching criterion for a given treatment was done on days 4, 6, 8, and 10. Significant effects were observed in the pups from the CP-treated groups on days 8 and 10 ($P < .05$); these effects were not due to differences between litters within treatments (Kruskal-Wallis one-way analysis of variance); S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).
12. Saline × saline progeny were compared statistically with CP × CP or CP × saline F_1 pups by using the *t*-test.
13. C. Bagley *et al.*, *Cancer Res.* **33**, 226 (1973).

15 May 1980; revised 22 July 1980

β-Endorphin: Possible Involvement in the Antihypertensive Effect of Central α-Receptor Activation

Abstract. *Clonidine and L-α-methylnoradrenaline (but not D-α-methylnoradrenaline) increase the release of a substance with β-endorphin immunoreactivity from slices of brainstem of spontaneously hypertensive rats, but not that of normotensive rats. It was reported earlier that opiate antagonists inhibit the hypotensive action of clonidine and α-methyldopa in spontaneously hypertensive but not in normotensive rats and that β-endorphin has hypotensive effects of its own. Together, these findings indicate that release of β-endorphin by central α-receptor agonists may contribute to the antihypertensive action of these drugs.*

Opioid peptides have been implicated in the control of pain sensation, mood, and various behavioral functions (*1*). Less attention has been devoted to the possible role of these substances in the central control of cardiovascular function. Morphine and some endogenous opiates can reduce blood pressure and heart rate, effects that result partly from a reduction of sympathetic tone as opiate receptors in the medulla oblongata are activated (*2, 3*). Clonidine, an antihypertensive drug that activates α-adrenergic receptors (α-receptors) in the same brain

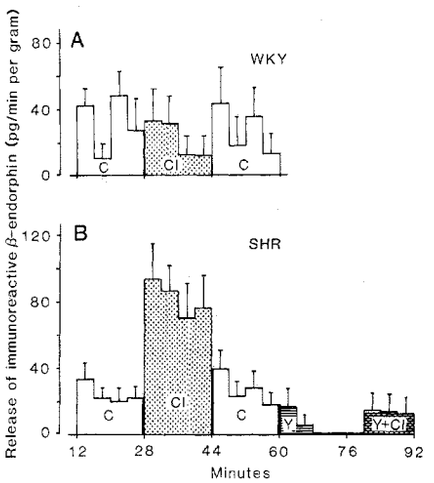

Fig. 1. The effect of clonidine on the release of β-endorphin immunoreactivity from slices of brainstem from (A) normotensive (*WKY*) and (B) hypertensive rats (*SHR*). The concentration of β-endorphin immunoreactive material in the lyophilized samples (*11*) was measured by radioimmunoassay (New England Nuclear) with ^{125}I-labeled human β-endorphin as tracer and a rabbit antibody to human β-endorphin. The antibody does not significantly cross-react with α-endorphin, α-melanocyte-stimulating hormone, leucine enkephalin or methionine enkephalin (< 0.01 percent), whereas there is 50 percent cross-reactivity with β-lipotropin. Bovine β-endorphin, which is identical to rat β-endorphin (*20*) had 14.3 ± 2.0 percent cross-reactivity at 50 percent displacement. Since cross-reactivity progressively decreased with increasing concentrations of bovine β-endorphin, it is probable that the increases we detected in human β-endorphin immunoreactivity reflect much greater increases in the release of the homologous rat β-endorphin. Although a contribution of β-lipotropin to the immunoreactivity measured is possible, this may be minimal in view of findings that in rat brain β-lipotropin represents a negligible fraction of the total β-endorphin immunoreactivity (*14*). Lyophilized samples were reconstituted in 0.2 ml of assay buffer. Standards prepared in assay buffer were supplemented with the same amount of salts present in the experimental samples. With this sample matrix, blanks bound 9.0 ± 0.9 percent and "zero" standards bound 30.8 ± 1.6 percent of the total radioactive material. The useful range of the standard curve was between 10 and 500 pg of human β-endorphin equivalent per assay tube. All incubations were done in siliconized test tubes. Separation of bound from free ligand was achieved by adsorption of free β-endorphin onto activated charcoal and centrifugation. Radioactivity in the supernatant was measured in a gamma counter. A value of zero was arbitrarily assigned to those control samples for which β-endorphin immunoreactivity was below the limit of detectability. Columns and bars represent means and standard errors from three experiments in WKY and 11 experiments in SHR. The concentration of clonidine (*Cl*) was $10^{-8}M$, that of yohimbine (*Y*) $10^{-6}M$. Open bars indicate control (*C*) values. In separate experiments (*10*), we tested the interaction of clonidine (5 μg/kg, intravenously) and naloxone (2 mg/kg, intraperitoneally) on systolic blood pressure of unanesthetized SHR and WKY. In seven WKY, clonidine reduced blood pressure from 125.2 to 113.8 mm-Hg before and from 130.0 to 117.6 mm-Hg after naloxone was given ($P > .5$). In contrast, in six SHR, the effect of clonidine (from 196.0 to 172.5 mm-Hg) was nearly abolished by naloxone (from 194.8 to 192.7 mm-Hg, $P < .001$).

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area, has effects similar to those of morphine. Clonidine reduces blood pressure and heart rate (*4*), can produce analgesia (*5*), and its withdrawal elicits symptoms similar to those of opiate withdrawal (*6*). Moreover, clonidine reverses symptoms of opiate withdrawal in addicts (*7*), whereas drugs that inhibit the interaction of clonidine with α-receptors, such as yohimbine and piperoxan, can elicit symptoms resembling opiate withdrawal (*8*). These similarities suggest some interaction between central opiates and the α-receptor systems.

We recently reported that naloxone inhibited the hypotensive effect of the central α-receptor agonists clonidine and α-methyldopa in spontaneously hypertensive rats (SHR) (*9*). Since clonidine and naloxone did not cross-react with each other's binding sites in the brain (*9*), the observed functional antagonism could be due to the release of an endogenous opiate by activation of α-receptors that participate in the central control of blood pressure. More recently, we found that the small hypotensive effect of clonidine and α-methyldopa in normotensive Wistar-Kyoto rats (WKY) was not antagonized by opiate antagonists (*10*). This indicates that the mechanism postulated to exist in SHR is either absent or inactive in WKY. We now provide direct evidence that a substance with β-endorphin immunoreactivity is released from the brainstem of SHR when central α-receptor agonists are administered. A similar release could not be detected in preparations from WKY.

Spontaneously hypertensive male rats of the Okamoto-Aoki strain were matched for sex and weight (300 to 350 g) with normotensive WKY rats. Brainstem slices were prepared and superfused with oxygenated Krebs-Henseleit solution at 37°C (*11*). The concentration of immunoreactive β-endorphin in consecutive 4-ml samples of the superfusate was measured by radioimmunoassay (Fig. 1). In three separate experiments in each of which the pooled brainstem slices from two WKY rats were used, β-endorphin concentrations during the control perfusion period were around the lower limit of detectability and were not increased by the presence of $10^{-8}M$ clonidine in the superfusing medium (dotted area in Fig. 1A). In 11 experiments with preparations from SHR (Fig. 1B), $10^{-8}M$ clonidine significantly increased β-endorphin concentrations to two or three times the control values, and this increase was reversed when the clonidine-free medium was again perfused. In two additional experiments, not shown here, a second exposure to the same concentration of clonidine produced an increase in β-endorphin similar to that seen during the first exposure period.

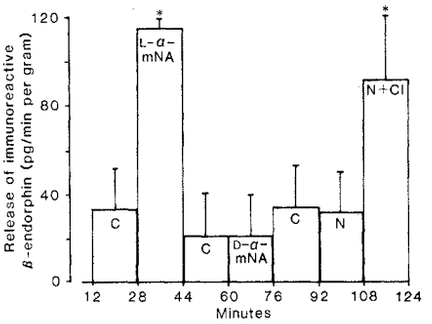

Fig. 2. Release of β-endorphin immunoreactivity by L- and D-α-methylnoradrenaline and clonidine (in the presence of naloxone) from slices of brainstem from hypertensive rats. For experimental protocol see (*11*) and Fig. 1. Columns represent means and standard errors from four experiments. Values were calculated from the cumulated release over the 16-minute perfusion periods. The concentration of L- and D-α-methylnoradrenaline (α-*mNA*) and naloxone (*N*) was $10^{-7}M$, and the concentration of clonidine (*Cl*) $10^{-8}M$. Asterisk indicates significant difference ($P < .05$) from the control value (*C*) (for L-α-methylnoradrenaline), or from the value obtained with naloxone alone (for clonidine plus naloxone).

In six of the 11 experiments shown in Fig. 1, superfusion was continued after the second control period with medium containing $10^{-6}M$ yohimbine, a potent α_2-receptor antagonist. In unanesthetized SHR we earlier found that yohimbine at 1 mg/kg completely eliminated the hypotension and bradycardia produced by clonidine (5 μg/kg) (*10*). Superfusion of brainstem slices from SHR with yohimbine (60 to 76 minutes) reduced somewhat the amount of β-endorphin released and prevented the increase in release by $10^{-8}M$ clonidine (76 to 92 minutes). These findings indicate that activation of α_2-receptors in the perfused brain region results in the release of a substance with β-endorphin immunoreactivity.

α-Methylnoradrenaline is a potent agonist of central α-receptors, and it is believed to mediate the antihypertensive action of α-methyldopa, from which it is formed in noradrenergic nerve terminals in the brain. We therefore tested the effect of L-α-methylnoradrenaline and of its inactive D-stereoisomer on β-endorphin release in brainstem slices of SHR. In four separate experiments, superfusion with $10^{-7}M$ L-α-methylnoradrenaline produced a highly significant, threefold increase in β-endorphin release, whereas the same concentration of D-α-methylnoradrenaline was without any effect (Fig. 2). In the same preparations, the effect of naloxone on the clonidine-induced release of β-endorphin was also tested. Naloxone itself ($10^{-6}M$) did not influence the amount of β-endorphin in the superfusate (92 to 106 minutes), and in the presence of naloxone, $10^{-8}M$ clonidine increased the release of β-endorphin to the same extent (106 to 120 minutes) as in its absence (see Fig. 1). This finding eliminates the possibility that naloxone antagonizes the effect of clonidine in the intact animal by inhibiting the release rather than the action of the endogenous opiate. It was earlier reported that naloxone can inhibit a morphine-induced increase in the opiate activity of cerebrospinal fluid in mice (*12*).

Our results, together with our earlier observations (*9*), strongly suggest that release of a β-endorphin-like material somewhere in the brainstem contributes to the antihypertensive action of central α-receptor stimulants in SHR. The inhibition of the hypotensive effects of clonidine and α-methyldopa by naloxone or naltrexone is accordingly due to the blocking of the effect of this opiate on opiate receptors inhibitory to sympathetic activity. β-Endorphin is present in neurons in the brain (*13*, *14*), and its calcium-dependent release by depolarizing stimuli (*15*) suggests that it may be a neurotransmitter. Also, β-endorphin administered centrally (*3*) or systemically (*16*) produces bradycardia and hypotension, effects similar to those of clonidine.

The failure of clonidine to release β-endorphin in brainstem slices from WKY correlates with the failure of naloxone to antagonize the hypotensive effects of clonidine and α-methyldopa in these animals (*10*) and also agrees with a reported failure of naloxone to antagonize the effects of clonidine in normotensive humans (*17*). These observations indicate that the mechanism postulated to explain various effects of clonidine in SHR cannot be generalized to different physiological conditions.

The mechanism of action of clonidine appears to be different in the two genetically matched rat strains. Destruction of the nucleus of the solitary tract (NTS) abolishes the hypotensive effect of clonidine in SHR, but does not influence the much smaller effect in WKY (*18*). It is possible that the release of endorphin-like substance occurs either in the NTS or in a neuronal pathway involving the NTS. Although the morphological relationship between central α-receptors and the exact locus of β-endorphin release is not known, the fact that the effect of

clonidine was demonstrated in relatively small brainstem fragments suggests that the two sites are anatomically close to each other and may even be in the same cell. Whether such an "opioidergic" pathway is limited to the SHR or has implications for the development of hypertension in general is still unclear. Findings of a close correlation between the level of blood pressure and pain threshold in various forms of hypertension (*19*) suggest a link between some endogenous opiates and the hypertensive process.

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References and Notes

1. A. J. Kastin, R. D. Olson, A. V. Schally, D. H. Coy, *Life Sci.* **25**, 401 (1979); H. H. Loh, L. F. Tseng, E. Wei, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2895 (1976); L. Graf, J. I. Szekely, A. Z. Ronai, Z. Dunai-Kovacs, S. Bajusz, *Nature (London)* **263**, 240 (1976); F. Bloom, D. Segal, N. Ling, R. Guillemin, *Science* **194**, 630 (1976).
2. M. Laubie, H. Schmitt, J. Canellas, J. Roquebert, P. Demichel, *Eur. J. Pharmacol.* **28**, 66 (1974); E. Freye, J. O. Arndt, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **307**, 123 (1979).
3. M. Laubie, H. Schmitt, M. Vincent, G. Remond, *Eur. J. Pharmacol.* **47**, 67 (1977).
4. W. Hoefke, W. Kobinger, *Arzneim. Forsch.* **16**, 1038 (1966); H. Schmitt, H. Schmitt, J. R. Boissier, J. F. Giudicelli, *Eur. J. Pharmacol.* **2**, 147 (1967).
5. S. Fielding, J. Wilker, M. Hynes, M. Szewczak, W. J. Novick, H. Lal, *J. Pharmacol. Exp. Ther.* **207**, 899 (1978); H. Schmitt, J.-C. Le Douarec, N. Petillot, *Neuropharmacology* **13**, 289 (1974).
6. J. I. Reid, H. J. Dargie, D. S. Davies, L. M. H. Wing, C. A. Hamilton, C. T. Dollery, *Lancet* **1977-I**, 1171 (1977).
7. M. S. Gold, D. E. Redmond, Jr., H. D. Kleber, *ibid.* **1978-II**, 599 (1978).
8. S. H. Snyder, *Nature (London)* **279**, 13 (1979).
9. C. Farsang and G. Kunos, *Br. J. Pharmacol.* **67**, 161 (1979).
10. C. Farsang, M. D. Ramirez-Gonzalez, L. Mucci, G. Kunos, *J. Pharmacol. Exp. Ther.* **214**, 203 (1980).
11. Rats were decapitated and the brains were quickly removed, leaving the pituitary behind in the scella. The brainstem was separated from the hemispheres and the cerebellum and was cut into 260-μm slices by a tissue chopper (McIlwain). Pooled slices from two rats were incubated for 20 minutes in 50 ml of Krebs-Henseleit buffer gassed with 5 percent CO_2 in O_2 at 37°C (pH 7.4). The slices (0.5 to 1 g, wet weight) were then transferred into a 1-ml superfusion chamber. Oxygenated buffer at 37°C was perfused at a rate of 1 ml/min and collected for periods of 4 minutes into ice-cold siliconized test tubes containing 0.2 ml of 1*N* HCl. The first three samples were discarded. Four samples were then collected as controls, and the superfusion medium was replaced by buffer containing various drugs as indicated in the text. Control and drug perfusion periods were repeated one or two more times. Immediately after their collection the samples were incubated at 95°C for 16 minutes, then frozen, lyophilized, and kept at −60°C until the assay. At the end of the experiment the brain slices were centrifuged for 1 minute at 12,000*g* to determine packed wet weight.
12. T. C. Fu, S. Halenda, L. Lawrence, T. T. Chau-Pham, B. R. Martin, W. L. Dewey, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 364 (Abstr.) (1979).
13. J. Rossier, T. M. Vargo, S. Minick, N. Ling, F. E. Bloom, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 562 (1977).
14. N. Ogawa *et al.*, *Life Sci.* **25**, 317 (1979).
15. H. Osborne, R. Przewlocki, V. Hollt, A. Herz, *Eur. J. Pharmacol.* **55**, 425 (1979).
16. I. Lemaire, R. Tseng, S. Lemaire, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6240 (1978).
17. M. J. Brown, C. T. Dollery, G. A. Fitzgerald, J. Watkins, C. Zamboulis, *Br. J. Clin. Pharmacol.* **9**, 302P (1980).
18. R. W. Rockhold and R. W. Caldwell, *Neuropharmacology* **18**, 347 (1979).
19. N. Zamir and M. Segal, *Brain Res.* **152**, 521 (1979).
20. N. G. Seidah, G. Gianoulakis, P. Crine, M. Lis, S. Benjannet, R. Routhier, M. Chretien, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3153 (1978).
21. The L- and D-α-methylnoradrenaline used in this study were donated by Sterling-Winthrop Corporation; clonidine and naloxone were gifts from Boehringer-Ingelheim and Endo Laboratories, respectively. The bovine β-endorphin used to determine cross-reactivity was a gift from N. G. Seidah. This work was supported by grants from the Medical Research Council of Canada.

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Thermoregulatory Significance of Thoracic Lobes in the Evolution of Insect Wings

Abstract. *The evolution of broadly attached thoracic lobes could have increased the body temperature excess of ancient wingless insects by 55 percent over that of lobeless forms. The subsequent expansion of these thoracic lobes for behavioral thermoregulation could have provided the morphological stage required for the evolution of functional wings.*

Many entomologists have speculated on the structural origin of insect wings, the possible selective pressures which produced them, and their sequential modification in the development of flight. Although most entomologists adhere to the "paranotal lobe" theory (*1*), which postulates that insect "pro-wings" were derived from nonarticulated lateral extensions of the thoracic terga (*2, 3*), reinterpretation of the juvenile wings of Paleozoic nymphs (*3*) suggests that their nymphal pro-wings were articulated structures which secondarily lost their movability by fusion with the tergum, and thus became evolutionarily convergent with "paranota" (Fig. 1).

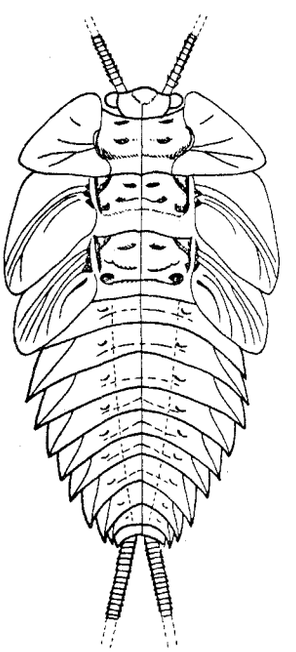

Fig. 1. Young nymph of the Paleozoic terrestrial palaeodictyopteran, *Rochdalia parkeri*, illustrating "articulated" thoracic lobes (*4*). Original reconstruction [After R. J. Wooten (*24*); from *Journal of Morphology*; reproduced with permission from the Wistar Institute].

A second major controversy has centered around the possible functions of these small, lobelike pro-wings, whether articulated or not, which likely served as the intermediate stage in the evolution of truly functional wings. These broadly attached pro-wings (hereafter termed "thoracic lobes") may have functioned originally to cover the spiracular openings or gills in amphibious ancestors (*3*), to protect and conceal insects from predators (*4*), to facilitate passive aerial dispersal by small insects (*5*), or to aid in sexual displays (*6*).

However, my experiments suggest that a more probable reason for the continued expansion of these small thoracic lobes to a critical size required for efficient gliding or flapping flight may have been their prior adaptation for behavioral thermoregulation. Because the activity of most insects is highly dependent on body temperature and the thermal conditions of their microhabitat (*7-11*), any thoracic structure that permitted the leg muscles of ancestral pterygotes (*12*) to heat rapidly and attain a higher equilibrium temperature would enhance locomotor efficiency, a condition crucial to insect survival and reproductive success (*13*).

Colias eurytheme butterflies were chosen to test this thermoregulatory hypothesis because their wings could be physically altered to approximate those of the ancestral pterygotes (*14*) and because their body length is near that of winged Protorthoptera (Paraplecoptera) with Thysanura-like immatures (*15*). In addition, preliminary temperature mapping of butterfly wings and analyses of energy exchange between wings and thorax in these butterflies showed that only the basal third of the wings were of ther-

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