Reports

Serotonin Antagonism of Noradrenaline in vivo

We have previously reported that pretreatment with serotonin reduces the mortality of mice given bacterial endotoxin (*1*). Since the administration of endotoxin is followed by the secretion of, and hypersensitivity to, the adrenalines (*2*), the possibility that serotonin might prove to have antiadrenergic properties was investigated. We have been able to demonstrate that serotonin antagonizes the effect of noradrenaline in four in vivo test systems.

Inhibition of acute noradrenaline toxicity in mice. Serotonin (250 to 450 μg) (*3*) was given to adult male mice (Carworth Farms) averaging 24 g in weight, in 0.1 ml of isotonic saline, divided between the intraperitoneal and subcutaneous routes. Fifteen minutes later 400 μg of noradrenalin were injected intravenously. Control mice, pretreated with saline, were similarly injected with noradrenaline. Serotonin pretreatment reduced the 1-hour noradrenaline mortality from 27/30 to 5/30, and in separate experiments was found to abolish the concomitant pulmonary edema caused by noradrenaline.

Suppression of the mouse pilomotor response. The erector response of the pilomotor muscles may be elicited by the injection of exogenous noradrenaline, or by the release of endogenous noradrenaline following exposure to cold or the injection of reserpine (*4*). We have elicited this reaction by the intramuscular injection of 100 μg of noradrenaline, the intraperitoneal injection of 50 μg of reserpine, or placement of the mouse on a cold metal plate at 1°C. All three methods evoked a response that was reproducible and easy to identify visually. Injection of 250 μg of serotonin intramuscularly 10 minutes before the experiment did not, in itself, cause piloerection and completely suppressed the piloerector response to all three forms of test stimulation. One hundred micrograms of the antiadrenergic compound dibenzyline, when injected intramuscularly, also blocked piloerection, but mecholyl was ineffective.

Lysis of small vessel tone. The local effect of serotonin upon foreleg small vessel resistance was studied in dogs anesthetized with pentobarbital (*5, 6*). A blood pump interposed between the femoral and brachial arteries maintained foreleg blood flow at a constant rate. Pressures were measured simultaneously, with transducers, in the brachial artery distal to the pump, in a footpad small artery, a paw small vein, and the cephalic vein. Large artery, small vessel (mainly arteriolar), and large vein resistances were separately calculated. In 32 preparations, small vessel neurogenic tone was varied by bilateral vagotomy, foreleg nerve section, and the administration of phentolamine. The administration of 4.5 μg of serotonin per minute, by constant infusion, or of 1.0 μg by instantaneous injection, into the brachial artery decreased small vessel resistance at all levels of tone. The decrement was proportional to the initial level of tone (Fig. 1). In seven other large dogs, after foreleg nerve section, increases in small vessel tone were induced by the infusion of 0.25 to 5.0 μg of noradrenaline per minute into the brachial artery. Infusion of 4.5 to 9.0 μg of serotonin during the noradrenaline infusion decreased elevated small vessel tone in these animals as well.

Inhibition of noradrenaline-induced bradycardia. Continuous heart rate measurements were obtained on pentobarbital-anesthetized dogs given an instantaneous intravenous injection of 10 μg of noradrenaline before and during an intravenous infusion of 300 μg of serotonin per minute.

Noradrenaline, when injected alone, decreased the heart rate during the first 15 seconds by an average of 15 percent. When it was administered during serotonin infusion, noradrenaline increased the heart rate by an average of 15 percent. We have also noted that the tachycardia induced by decreasing the intraluminal pressure of the carotid sinus is abolished during serotonin infusion. These results are similar to the findings of Heymans (*7*) that serotonin, when painted on the carotid sinus, abolishes the sensitivity of the sinus to pressure changes. Since norepinephrine-induced bradycardia is dependent on the carotid sinus reflex and since the denervated heart responds to norepinephrine with tachycardia (*8*), our results are interpreted to mean that serotonin antagonizes the action of norepinephrine upon the carotid sinus.

Our observations that serotonin antagonizes the effects of noradrenaline on four different in vivo test systems suggest strongly that a biological role of serotonin lies in its interaction with the adrenalines. In vitro studies showing that the adrenalines inhibit serotonin-induced contraction of rat uterine smooth muscle (*9*) permit a similar conclusion. Such work emphasizes the negative aspects of the interaction. However, Thomas (*10*) has presented evidence for positive interaction, noting that serotonin enhances skin necrosis due to adrenaline, and Zweifach (*11*) has described a biphasic influence of serotonin on the response to adrenaline in the blood vessels of the rat mesoappendix.

The mechanisms involved in the interaction of these hormones are not clear. The hormones may interact at an organ level through interrelated physiological systems, or perhaps at a molecular level. If the latter occurs, it may be possible to reconcile the above conflicting findings. The in vitro observations of Furchgott (*12*) suggest that serotonin and the adrenalines can compete for biological action sites; thus inhibition might be accounted for on a competitive basis. On the other hand, serotonin and the adrenalines are catabolized by monoamine oxidase (*13*) and ceruloplasmin (*14*); competition for the destructive enzymes might account for potentiation.

Two of our test systems deal with aspects of the cardiovascular system.

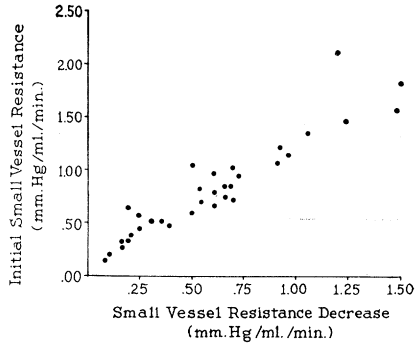

Fig. 1. Serotonin depression of limb small vessel resistance at varying initial levels of neurogenic tone. Serotonin was infused into the brachial artery at the rate of 4.5 μg/min. Thirty-five tests in 22 animals. Initial tone levels were spontaneous or experimentally modified by nerve section high in the limb. Measurements were steady-state.

All technical papers are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in *Science* 125, 16 (4 Jan. 1957).

Serotonin antagonism of the effects of noradrenaline may explain certain of the unusual cardiovascular actions of the former compound. Thus, intravenous serotonin is reported to cause systemic hypotension in neurogenic hypertensive animals and hypertension in neurogenic hypotensive animals (*15*). Similarly, in the perfused dog limb, serotonin increases the net resistance across the vessels in limbs with low neurogenic vessel tone and decreases resistance when the initial tone is high (*6, 16*). Apparently, in these preparations, the direction of systemic pressure and peripheral resistance responses to serotonin depends, at least in part, upon initial neurogenic tone.

The reason for this tone dependence in the dog forelimb has been demonstrated to lie in the differing responses of small and large vessel segments to serotonin. Serotonin constricts the large arteries and veins and dilates small vessels (*5, 6*). The magnitude of constriction in large vessels is largely independent of the initial tone, whereas the dilatation in small vessels is directly proportional to the level of initial neurogenic tone. The addition of a fixed, large-vessel constriction to a small vessel dilatation which increases with increasing tone results in a total resistance change which may be dilator or constrictor, depending upon the initial neurogenic tonic input to the small vessels.

The tone of small vessels is in part related to the concentration of noradrenaline at the nerve endings surrounding them. We suggest that serotonin may decrease small vessel tone through its capacity to antagonize the vasoconstrictor activity of noradrenaline on the small vessels. The diverse effects of serotonin upon total resistance changes in the perfused dog limb and upon systemic blood pressure can be explained similarly.

Serotonin and noradrenaline are located in the same areas of the central nervous system (*17*). Noradrenaline depletion (*4, 18*) and the release of bound serotonin (*19*) from the brain stem have both been suggested as mechanisms for reserpine tranquilization. Extension of noradrenaline-serotonin antagonism to a hypothetical critical area in the central nervous system allows reconciliation of the divergent hypotheses. Thus a functional norepinephrine deficiency might result at such a site if the action of available norepinephrine were inhibited by increases in free serotonin (*20*).

PAUL GORDON
FRANCIS J. HADDY
MORRIS A. LIPTON

Veterans Administration Research Hospital, Chicago, Illinois, and *Departments of Pharmacology, Physiology,* and *Medicine, Northwestern University Medical School, Chicago*

References and Notes

1. P. Gordon and M. A. Lipton, *Federation Proc.* 16, 301 (1957).
2. L. Thomas, *Ann. Rev. Physiol.* 16, 467 (1954); *J. Exptl. Med.* 104, 865 (1956).
3. Serotonin creatinine sulphate (Sigma) and L-norepinephrine bitartrate (Mann) were employed in these experiments. The quantities given refer to the calculated free bases.
4. G. M. Everett, J. E. P. Toman, A. H. Smith, *Federation Proc.* 16, 295 (1957).
5. F. J. Haddy, M. Fleishman, D. A. Emanuel, *Circulation Research* 5, 247 (1957).
6. F. J. Haddy, P. Gordon, M. A. Lipton, D. A. Emanuel, J. Scott, *ibid.*, in press.
7. C. Heymans and G. Van Den Heuvel-Heymans, *Arch. intern. pharmaco-dynamie* 93, 95 (1953).
8. R. Greenberg and C. B. Lambeth, *Federation Proc.* 11, 59 (1952); A. A. Siebens, B. F. Hoffman, Y. Euson, J. E. Farrell, C. McC. Brooks, *Am. J. Physiol.* 175, 1 (1953).
9. A. H. Amin, T. B. B. Crawford, J. H. Gaddum, *J. Physiol. (London)* 126, 596 (1954).
10. L. Thomas, B. W. Zweifach, B. Benacerraf, *Trans. Assoc. Am. Physicians* 70, 54 (1957).
11. B. W. Zweifach and C. B. Metz, *J. Clin. Invest.* 34, 653 (1955).
12. R. F. Furchgott, *J. Pharmacol. Exptl. Therap.* 14, 280 (1954).
13. E. A. Zeller, J. Barsky, E. R. Berman, *J. Biol. Chem.* 214, 267 (1955).
14. B. E. Leach, M. Cohen, R. G. Heath, S. Martens, *A.M.A. Arch. Neurol. Psychiat.* 76, 635 (1956); C. C. Porter, D. E. Titus, B. E. Sanders, E. V. C. Smith, *Science* 126, 1014 (1957).
15. I. H. Page, *Am. J. Physiol.* 184, 265 (1956).
16. I. H. Page and J. W. McCubbin, *Circulation Research* 1, 354 (1953).
17. H. E. Himwich, *Science* 127, 59 (1958).
18. A. Carlsson, M. Lundquist, T. Magnusson, *Nature* 180, 4596 (1957).
19. P. A. Shore, A. Pletcher, E. G. Tomich, A. Carlsson, Ronald Kurtzman, B. B. Brodie, *Ann. N.Y. Acad. Sci.* 66, 609 (1957).
20. Dr. Haddy is a clinical investigator of the Veterans Administration. This investigation was aided by grant No. H-1967 from the National Heart Institute, National Institutes of Health, and by the Ciba Pharmaceutical Co.

14 April 1958

Auxin Action on Coleoptiles in the Presence of Nitrogen and at Low Temperature

Coleoptile elongation is thought to be influenced by the plasticity of the pectic matrix of the cell walls. It is also thought that methylation of pectin increases the wall plasticity by reducing the number of carboxyl groups which may be cross-linked by divalent cations. The balance between methylation by methyl transfer reactions and demethylation by pectin methylesterase (PME) controls the methyl content of pectin. Pectin methylesterase activity in the cell wall is probably reduced by the auxin-mediated binding of the enzyme demonstrated by Glasziou (*1*). Under conditions of active methylation, reduced PME activity would allow an increase in the total methyl content of pectin by reducing hydrolysis of methyl groups. This hypothesis accounts for the effects of auxin on methylation (*2*) and cell expansion. Since auxin-mediated binding of PME is thought to be an adsorption reaction (*1*) it is likely to be insensitive to metabolic conditions. It is also likely that PME activity is less sensitive to metabolic conditions than pectin methylation. The sensitivity of auxin-induced expansion to metabolic control may thus reflect the greater metabolic sensitivity of the methylation process. The results presented in this report are consistent with the above interpretation.

Dark grown wheat coleoptiles (var. Federation) were selected for uniformity of length (±0.1 cm) in the range 3.0 to 3.7 cm approximately 95 hours after sowing. Sections 2.2 cm in length were cut 3 mm behind the apex, the primary leaf was removed, and the sections were washed in aerated distilled water for 1 to 2 hours prior to treatment. The coleoptiles were treated for 90 minutes in a basal medium of distilled water or 0.02 molar calcium chloride in the presence or absence of 10 mg/liter of unbuffered β-indolylacetic acid (IAA). Each treatment was also carried out in nitrogen or at 2° to 3°C after prior equilibration of the solutions to these conditions and after prior washing of the sections for at least 10 minutes in distilled water equilibrated to either nitrogen or low temperature. After treatment, the sections were trimmed to 2 cm, and deformation under a constant load of 300 mg for 15 minutes was measured (*3*). All manipulations and deformation measurements for the nitrogen treatments were carried out in an atmosphere of nitrogen in a Perspex box fitted with Polythene sleeves. A refrigerated, constant-temperature room was used for the low-temperature treatments, manipulations, and measurements. Coleoptile expansion during each treatment was determined on ten 1-cm sections. A basal medium of calcium chloride solution was used to prevent expansion during treatment in air at 25°C. With turgid material which had not undergone differential expansion, changes in deformability were taken to reflect changes in wall properties.

Significant coleoptile expansion oc-

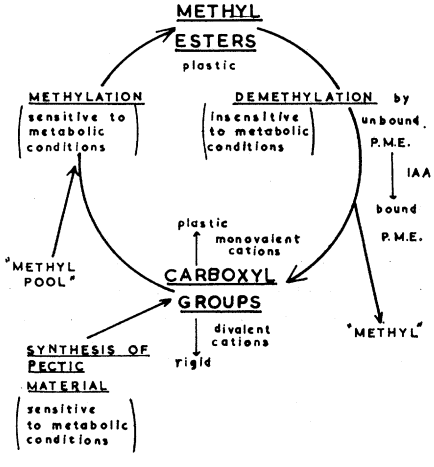

Fig. 1. Possible interconnections between methyl and carboxyl groups of the pectic substances.