g of tissue. This is within the range of values of cerebral blood flow obtained by other methods (*14*). Comparison of the counts from fixed and mobile fractions during the 20-second interval immediately following the completion of activation (determined by extrapolation) indicates that 63 percent of the induced activity washed out of the imaged volume and 37 percent remained immobile. As indicated in the discussion of the animal data, the immobile fraction is most likely due to the incorporation of ^{15}O in slowly diffusing cerebrospinal fluid and in bone, and also to chemical attachment of ^{15}O to tissue protein.

Preliminary data obtained from the application of the above technique to studies of perfusion in mammary tumors in C_3H mice and tumors (rhabdomyosarcoma) in WAG/Rij rats indicate, as expected, that the rate of perfusion varies widely from tumor to tumor. Our data also indicate that the rate of perfusion for a given tumor can be significantly affected by anesthetization of the animal subject. Such observations are consistent with those reported by other investigators (*15*).

We are developing the photon activation–^{15}O decay technique for the more precise, noninvasive measurement of regional tissue perfusion in animals under a variety of physiological and pharmacological stresses. We are also employing the technique to investigate perfusion rates in human tumors being treated with radiation. As applied to radiotherapy patients, this technique is truly noninvasive, since the radiation dose to the irradiated site is determined solely from therapeutic considerations. When the signal-to-dose ratio has been optimized by hardening the activating beam and by utilizing a positron camera for detection of pairs of annihilation photons, we anticipate extension of the photon activation–^{15}O decay technique to the study of other pathological conditions (such as cerebral ischemia) where the gravity of the prognosis will justify the radiation dose.

WALTER L. HUGHES
GILBERT H. NUSSBAUM
RAYMOND CONNOLLY
BAHMAN EMAMI
PAUL REILLY

Department of Therapeutic Radiology, Tufts-New England Medical Center, Boston, Massachusetts 02111

References and Notes

1. M. M. Ter-Pogossian, J. O. Eichling, D. O. Davis, C. C. Carter, in *Brain and Blood Flow: Proceedings of the Fourth International Symposium on the Regulation of Cerebral Blood Flow*, R. W. Ross Russell, Ed. (Pitman, London, 1971), pp. 1–4.
2. Y. L. Yammamoto, C. Thompson, E. Meyer, J. Little, *J. Comput. Assist. Tomogr.* **1**, 43 (1977).
3. R. F. Donley, T. M. Sundt, Jr., R. E. Anderson, F. W. Sharbrough, *Stroke* **6**, 121 (1975).
4. M. Mantyla, J. Kuikka, A. Rekonen, *Br. J. Radiol.* **49**, 335 (1976).
5. E. Spring and T. Vayrynen, *Phys. Med. Biol.* **15**, 23 (1970).
6. E. Stranden, *ibid.*, **22**, 348 (1977).
7. G. W. Bennett, J. O. Archambeau, B. E. Archambeau, J. I. Meltzer, C. L. Wingate, *Science* **200**, 1151 (1978).
8. S. Graffman and B. Jung [*Acta Radiol. Ther. Phys. Biol.* **14**, 113 (1975)] have also shown that some of the ^{15}O produced by 175-MeV protons passing through tissue behaves as $H_2{}^{15}O$.
9. J. P. Woodcock, *Theory and Practice of Blood Flow Measurement* (Butterworth, London, 1975), pp. 158–165.
10. S. S. Kety, in *Methods in Medical Research*, H. Bruner, Ed. (Year Book, Chicago, 1960), vol. 8.
11. For the geometry, electronic settings, and apparatus employed, the overall photon-pair detection efficiency was about 1 percent, and the volume resolution was approximately 5 cm^3.
12. S. S. Kety and C. F. Schmidt, *Am. J. Physiol.* **143**, 53 (1945).
13. One of three fields utilized for delivering the 45-MV x-ray dose. In fact, only a minor portion of the total patient dose was from this source of radiation. The majority of the dose was delivered by cobalt-60 gamma rays.
14. J. P. Woodcock, *Theory and Practice of Blood Flow Measurement* (Butterworth, London, 1975), p. 196.
15. G. D. Zanelli, P. B. Lucas, J. F. Fowler, *Br. J. Cancer* **32**, 380 (1975).
16. The scintillation detectors and coincidence counting electronics used to obtain the data presented in Fig. 1 were lent by Dr. G. W. Bennett of Brookhaven National Laboratory.

30 October 1978

Effect of Fluorine Substitution on the Agonist Specificity of Norepinephrine

Abstract. *Substitution of fluorine for hydrogen in position 2, 5, or 6 of the aromatic ring of norepinephrine markedly alters the α- and β-adrenergic agonist properties of norepinephrine. The 6-fluoro isomer is an α-adrenergic agonist with virtually no β agonist activity, while the 2-fluoro isomer is a β-adrenergic agonist with little α activity. The 5-fluoro isomer is equipotent with norepinephrine as an α agonist and significantly more potent as a β agonist. The possible physiochemical basis for these differences is discussed.*

We have been investigating the effects of fluorine substitution on the aromatic ring in a series of ring-hydroxylated biogenic amines. Our hope was that increased phenolic pK_a, altered hydrogen-bonding properties, and enhanced lipophilicity would produce altered but ultimately predictable behavior with respect to transport mechanisms, enzyme active sites, and receptor recognition. To this end, we prepared a series of ring-fluorinated tyramines (*1*), dopamines (*1*), and 5-hydroxytryptamines (*2*). These analogs were evaluated in a number of systems, and the results suggested that they will be of considerable value as biochemical and pharmacological tools (*3*). We therefore extended our synthetic efforts and recently synthesized the three isomeric ring-fluorinated analogs of norepinephrine (NE), the primary adrenergic transmitter. We report that the site of fluorine substitution dramatically affects the specificity of these NE analogs and suggest that the present findings may have considerable significance regarding our knowledge of receptor-agonist interactions.

The synthetic route involved side-chain elaboration of the isomeric fluorinated 3,4-dimethoxybenzaldehydes prepared from the corresponding diazonium fluoroborates by our photochemical fluorination procedure (*1*). Cyanohydrin formation with trimethylsilyl cyanide (*4*), lithium aluminium hydride reduction, and boron tribromide demethylation afforded the desired ring-fluorinated (±) norepinephrines, isolated as the hydrochloride salt (*5*) (Fig. 1). The stability of the fluoroderivatives was similar to that of NE. Stock solutions in 0.001*M* HCl were kept at −20°C for several weeks without loss of biological potency.

The effect of fluorine substitution on the α-adrenergic agonist properties of (±)NE was determined in the isolated aortic strip of the guinea pig (Fig. 2A) and the β-adrenergic agonist properties in the isolated guinea pig atrial preparation (Fig. 2B) (*6, 7*). As indicated by the dose-response curve of aortic strip contraction (Fig. 2A), 5- and 6-fluoronorepinephrine were equipotent with (±)NE [median effective concentration (EC_{50}) = 2.6 μ*M*] as α-adrenergic agonists. The α-adrenergic activity of 2-fluoronorepinephrine (EC_{50} = 110 μ*M*) was marginal, with the compound exhibiting a potency only 0.03 times that of (±)NE. Pretreatment of the aortic strip with the α-adrenergic blocking agent phentolamine (10 μ*M*) completely blocked the response to both (±)NE and the fluorine derivatives, whereas pretreatment with the β-specific blocking agent propranolol (10 μ*M*) had no effect. The β agonist potencies are indicated by the dose response for the increase in rate of contraction of the atrial preparation shown in Fig. 2B. 2-Fluoronorepinephrine was equipotent with (±)NE (EC_{50} = 0.7 μ*M*) in this regard, whereas the 5-fluoro derivative (EC_{50} = 0.1 μ*M*) was approxi-

0036-8075/79/0615-1217$00.50/0

mately seven times more effective than (±)NE. The 6-fluoro derivative, in contradistinction to its α-adrenergic agonist potency, showed only a weak β-adrenergic agonist effect (EC_{50} = 72 μ*M*), estimated at only 0.009 times the potency of (±)NE. Pretreatment of the atrial preparation with propranolol (10 μ*M*) completely blocked the atrial response to all four catecholamines, whereas phentolamine (10 μ*M*) had essentially no effect on the response.

Pretreatment of either the aortic strip or the atrial preparation with an inhibitor of catecholamine uptake, desmethylimipramine (10 μ*M*), for 10 minutes potentiated the response to (±)NE and to the 5- and 2-fluoro derivatives but had no effect on the response to the 6-fluoro derivative. The observed potentiation of the action of 2- and 5-fluoronorepinephrine by desmethylimipramine presumably results from an increased local concentration of the fluoro derivatives as a consequence of desmethylimipramine inhibition of the NE uptake mechanism (*8*). The insensitivity of 6-fluoronorepinephrine to desmethylimipramine suggests relative inability of the uptake system to transport the 6-fluoro derivative into the axoplasm.

From the present evidence it is clear that fluorine substitution exerts a strong influence on the specificity of the interaction of NE with adrenergic receptors. Substitution of position 2 of NE with fluorine results in an almost pure β-adrenergic agonist, equipotent with the parent NE. However, this derivative has essentially no α-adrenergic activity. An opposite effect is seen with substitution of position 6 of NE with fluorine. Here the 6-fluoro derivative has predominately α-adrenergic agonist properties and displays little, if any, β-adrenergic activity. In the case of substitution on position 5, the fluoro derivative, like NE, displays both α- and β-adrenergic activity. However, while 5-fluoronorepinephrine is equipotent with NE as regards α-adrenergic activity, the β-adrenergic agonist potency is significantly greater than that of NE.

Understanding of the mechanism responsible for these apparent differences in the biological properties of the isomeric fluoro derivatives of NE will require further study. It is not clear whether the observed differences are due to variable access to the receptor, differences in binding affinity, or differences in the ability to initiate a response following binding. Preliminary evidence from specific receptor binding studies supports the hypothesis that fluorine substitution alters the specificity and affinity of NE receptor binding (*8*). It is attractive to speculate that electronic rather than steric factors are operative. A small van der Waals radius and a short bond length to carbon render fluorine sterically similar to hydrogen. The high electronegativity of this substituent, however, greatly alters the electron density distribution of the molecule (*9*). In the chiral NE molecule, three groups linked to the asymmetric benzyl carbon—the aminomethyl, the hydroxyl, and the aromatic ring bearing the two phenolic groups—are concerned with receptor binding (*10*). Thus, perturbations in the ionization of the phenolic groups of the fluorinated NE could alter agonist behavior. Spectrophotometrically determined pK_a values for 2-, 5-, and 6-fluoronorepinephrine are 7.8, 7.9, and 8.5, respectively (*11*), compared with 8.9 for NE (*12*). In the 5- and 6-fluoro analogs, the 4-hydroxyl group will be preferentially ionized, while the 2-fluoro substituent will most affect the acidity of the 3-hydroxyl group. Fluorine substitution also increases the lipophilicity of phenolic compounds, an increase apparently related to pK_a alterations (*13*). Thus, local lipophilic perturbations could also vary with the site of fluorine substitution.

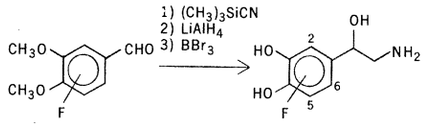

Fig. 1. Synthesis of fluorinated norepinephrines.

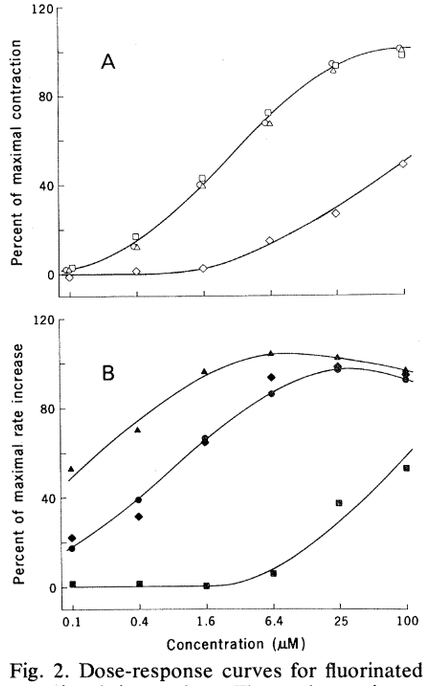

Fig. 2. Dose-response curves for fluorinated norepinephrine analogs. The α-adrenergic potency in the aortic strip (A) and β-adrenergic potency in the atrial preparation (B) were determined for (○, ●) (±)NE, (◇, ◆) 2-F-NE, (△, ▲) 5-F-NE, and (□, ■) 6-F-NE. Preparations removed from 250- to 300-g male Hartley guinea pigs were individually bathed in 10-ml chambers containing guinea pig Tyrode's solution (37°C) bubbled with 95 percent O_2, 5 percent CO_2. Tension and rate were monitored with a Grass model 79D polygraph and Grass FTO3C force displacement transducers. For the aortic strip, potency is expressed as the percentage of maximal (±)NE contraction. In the atrial preparation, it is expressed as the percentage of the maximal increase in contraction rate (PMRI): PMRI = I_{60}/I_{60max}, where I_{60} is the percentage of rate increase 60 seconds after dose addition and I_{60max} is the maximal I_{60} observed in the presence of (±)NE. Results are the means of eight experiments. Standard derivations in the range of the EC_{50} were not greater than 0.07 percent in the aortic strip and 0.08 percent in the atrial preparation. For the potency of 2-F-NE in the aorta and 6-F-NE in the atria, P = .002 and .001, respectively (paired, two-tailed t-test).

Initial infrared spectral studies have failed to detect such hydrogen bonding in the fluoronorepinephrines. However, recent studies with 2-, 5-, and 6-fluorodopamine by Goldberg *et al.* (*16*) demonstrated that fluorine substituents do not alter the agonist properties of dopamine. In addition to being equipotent with dopamine in the dopamine-specific renal blood flow preparation in the dog (*17*), these derivatives were equipotent with dopamine as α-adrenergic (*18*) and β-adrenergic (*19*) agonists. This lack of specificity in the dopamine derivatives shows that the specificity conferred by the *o*-fluoro substituent on the catechol ring is operative only when the β-hydroxyl group is present. This specificity presumably reflects fluorine-hydroxyl interactions or fluorine-induced electronic effects which are manifested only in a chiral molecule. It should also be noted that hydrogen bonding involving the β-hydroxyl group would not, by itself, provide a rationale for the increased β-adrenergic agonist activity of 5-fluoronorepinephrine. An understanding of the physiochemical basis for the specificities of these derivatives would provide an important insight into the nature

An alternative hypothesis requires hydrogen bonding between the β-hydroxyl group and an ortho-situated fluorine atom. Such interactions would favor different rotamers. To our knowledge, such hydrogen bonding in *o*-fluorobenzyl alcohols has not been reported, although nuclear magnetic resonance (NMR) evidence for hydrogen bonding in *o*-fluorobenzamides has been described (*14*), as well as rotamer stabilization through hydrogen bonding in aliphatic fluoro alcohols (*15*).

of the interaction of NE with specific binding sites on macromolecular systems.

Apart from mechanistic considerations, the agonist specificities of the "fluoronephrines" (*20*) make them extremely useful tools in neuropharmacology. The specific α-agonist properties of 6-fluoronephrine have been utilized to investigate the α-adrenergic component of the adenosine 3′,5′-monophosphate generating system in rodent cortex (*8*). The availability of an electron microscopic technique for visualizing fluorine in low concentrations in biological specimens may make it possible to actually localize a fluoronephrine-receptor complex at an ultrastructural level (*21*).

DANIELE CANTACUZENE
KENNETH L. KIRK
Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014
DAVID H. MCCULLOH
CYRUS R. CREVELING
Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases

References and Notes

1. K. L. Kirk, *J. Org. Chem.* **41**, 2373 (1976).
2. _____, *J. Heterocycl. Chem.* **13**, 1253 (1976).
3. C. R. Creveling, H. Seifried, D. Cantacuzene, K. L. Kirk, in *Transmethylation*, E. Usdin, R. Borchardt, C. Creveling, Eds. (Elsevier North-Holland, New York, 1979), pp. 269–276; H. Seifried, C. R. Creveling, D. Cantacuzene, K. L. Kirk, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, in press.
4. D. A. Evans, G. L. Carroll, L. K. Truesdale, *J. Org. Chem.* **39**, 914 (1974).
5. Purity and identity of all new compounds were established by combustion analysis, NMR, mass spectrometry, and thin-layer and high-pressure liquid chromatography (K. L. Kirk, D. Cantacuzene, C. R. Creveling, *J. Med. Chem.*, in press).
6. Department of Pharmacology, University of Edinburgh, *Pharmacological Experiments on Isolated Preparations* (Churchill Livingstone, New York, ed. 2, 1970), pp. 96–97 and 112–113; H. Blaschko and E. Muscholl, Eds., *Handbook of Experimental Pharmacology* (Springer-Verlag, New York, 1972), vol. 33, pp. 283–335.
7. J. E. Wikberg, *Nature (London)* **273**, 164 (1978).
8. J. W. Daly, W. Padgett, Y. Nimitkitpaisan, D. Cantacuzene, K. L. Kirk, C. R. Creveling, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 533 (1979).
9. P. Goldman, *Science* **164**, 1123 (1969).
10. P. N. Patil, J. B. LaPidus, A. Tye, *J. Pharm. Sci.* **59**, 1205 (1970).
11. Ionization constants for the fluoronorepinephrines were determined spectrophotometrically (*1*), using argon-flushed solutions.
12. B. Martin, *J. Phys. Chem.* **75**, 2657 (1971); G. P. Lewis, *Br. J. Pharmacol.* **9**, 488 (1954).
13. T. Fujita, J. Iwasa, C. Hansch, *J. Am. Chem. Soc.* **86**, 5175 (1964).
14. H. Fritz and T. Winkler, *Helv. Chim. Acta* **57**, 836 (1974).
15. P. J. Krueger and H. D. McHee, *Can. J. Chem.* **42**, 326 (1964).
16. L. I. Goldberg, J. D. Kohli, K. L. Kirk, C. R. Creveling, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 601 (1979).
17. L. I. Goldberg, P. K. Volkman, J. D. Kohli, *Annu. Rev. Pharmacol. Toxicol.* **18**, 15 (1978).
18. J. L. McNay and L. I. Goldberg, *J. Pharmacol. Exp. Ther.* **151**, 23 (1966).
19. R. H. McDonald and L. I. Goldberg, *ibid.* **140**, 60 (1963).
20. The term fluoronephrine was suggested by J. Hanig.
21. J. L. Costa, D. C. Joy, D. M. Maher, K. L. Kirk, S. W. Hui, *Science* **200**, 537 (1978).

30 October 1978; revised 29 January 1979

Embryonic Development of Identified Neurons: Temporal Pattern of Morphological and Biochemical Differentiation

Abstract. *Individually identified neurons can be recognized in grasshopper embryos, and are accessible to examination by morphological, physiological, and biochemical techniques from their birth to their maturation. Only after the axon of an identified neuron reaches its postsynaptic target does the neurotransmitter accumulate, the soma rapidly enlarge, and the central arborizations greatly expand.*

Our understanding of neuronal structure and function and of the cellular basis of behavior have profited immensely by the study of identified neurons (*1*). Identified neurons are unique cells that can be repeatedly recognized from animal to animal within the same species; such neurons have a characteristic morphology, physiology, and biochemistry. The embryonic development of these characteristic properties can potentially be examined from the birth of the neuron to its maturation. A prerequisite for such a study is the selection of a species with identified neurons that are relatively large during embryonic development and are accessible to a variety of cellular techniques.

We have been studying the development of a group of identified neurons in the embryonic central nervous system (CNS) of the grasshopper. These neurons are individually identifiable and accessible from their birth to their maturation (*2*). In this report we describe the morphological features of an identified neuron that are temporally associated with the appearance of its neurotransmitter. The cell lineage and developmental timetable of the morphological and physiological properties of these neurons are described elsewhere (*2*).

The subjects of this study are the dorsal unpaired median (DUM) neurons of the grasshopper (*3–5*). Their cell bodies (somata) form a distinct cluster on the dorsal midline of each segmental ganglion. They are termed unpaired because, unlike other neurons in the ganglion, those DUM cells thus far identified do not occur in bilaterally symmetrical pairs. Each DUM neuron soma gives rise to a median neurite which bifurcates into bilaterally symmetrical processes that in some cases exit the ganglion through peripheral nerve roots and innervate skeletal muscles. The largest individual DUM neuron in the third thoracic ganglion (soma diameter, ~ 60 μm) has axons emerging bilaterally in nerve 5 and innervates the extensor tibiae muscles of the left and right hindlegs (this neuron is therefore called Dumeti) (*4*). The neurotransmitter of Dumeti is the biogenic amine octopamine (*5*). The somata of the DUM neurons stain with neutral red (*5*), a vital dye that selectively stains monoamine-containing neurons in the leech (*6*) and lobster (*7*); evidence presented below suggests that octopamine is the transmitter for most, if not all, of the DUM neurons. Each thoracic ganglion contains about 80 DUM neurons; only six to eight of them have large-diameter somata like Dumeti, whereas most are somewhat smaller (15 to 30 μm in diameter). The only identified DUM neuron for which we know the peripheral target is Dumeti. Although the target is a muscle, this neuron is not a motoneuron but rather modulates both neuromuscular transmission and the responsiveness of the muscle (*5*).

Embryonic development in the grasshopper *Schistocerca nitens* takes 20 days at 35°C (*8*). Maintenance of a colony of the animals ensures a year-round supply of embryos. The adult CNS, consisting of a brain and a chain of ventral segmental ganglia, develops chiefly from enlarged ectodermal cells (neuroblasts) which constitute the precursor cells for most of the central neurons (*9, 10*). The three adult thoracic segmental ganglia (T_1, prothoracic; T_2, mesothoracic; and T_3, metathoracic) each contain approximately 3000 neurons which are born and largely differentiate during the 20 days of embryogenesis (*11*). By contrast to the DUM neurons, most of these neurons are bilaterally paired and have their somata on the ventral surface or lateral edge of the ganglion. These paired neurons develop from the left and right plates of ventral neuroblasts. There are about 30 neuroblasts in each ventral plate, making a total of 60 neuroblasts per segment (*10*).

Each segmental array of neuroblasts also contains a single unpaired neuroblast at the posterior end of the segment and dorsal to the paired plates of ventral neuroblasts (*9, 10*). The unpaired neuroblast gives rise to specific identified DUM neurons (*2*); it begins dividing about day 6 and degenerates about day 16. The progeny from the unpaired neuroblast extend anteriorly across the dorsal surface of the developing ganglion, with the oldest progeny furthest or most anterior from the unpaired neuroblast (*12*). This pattern of development leaves

0036-8075/79/0615-1219$00.50/0