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20. A 1-kb fragment of IAP DNA (Sst I–Bam HI) from clone IAP 81 [J. A. Moshier, R. A. Morgan, R. C. C. Huang, in *Interrelationships Among Aging, Cancer and Differentiation*, B. Pullman, Ed. (Reidel, Dordrecht, Holland, 1985), pp. 101–116] that was subcloned into phage M13 and termed p815/B1.0 was provided by R. C. C. Huang. The fragment was labeled with ^{32}P by the random primer method (*19*) to a specific activity of 1×10^8 to 5×10^8 cpm/μg.
21. High molecular weight genomic DNA was isolated from individual adult mice as follows: minced liver (0.4 to 0.5 g) was homogenized in a tight-fitting glass-Teflon homogenizer with 5 ml of ice-cold Iso-Hi-pH buffer [0.14 M NaCl, 0.01 M tris (pH 8.4), 1.5 mM $MgCl_2$] [U. Lindberg and J. E. Darnell, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 1089 (1970)] containing 0.1% NP-40 and then centrifuged at 3000 rpm in an HB-4 rotor at 4°C for 5 min. The supernatant and loose material over the pellet were removed by aspiration. The pellet was homogenized in 0.8 ml of Iso-Hi-pH buffer containing 0.1% NP-40 at 0°C. While vortex-mixing the resuspended nuclear pellet, we added an equal volume of ice-cold Iso-Hi-pH buffer containing 0.1% NP-40, 2% SDS, and proteinase K (1 mg/ml) (the latter solution is a slurry; SDS precipitates at this temperature). The resulting mixture was incubated at 37°C for 2 to 3 hours. The nucleic acids were gently extracted twice with 25:24:1 phenol:chloroform:isoamyl alcohol, followed by gentle extraction with 24:1 chloroform:isoamyl alcohol. The aqueous phase was dialyzed (Spectra/pore 2, Spectrum Medical Industries) first against 3 liters of 20 mM tris (pH 7.5) and 10 mM EDTA and then against 3 liters of 5 mM tris (pH 7.5) and 1 mM EDTA (with three changes of the latter dialyzing solution) at room temperature. The DNA samples were stored at 4°C for no longer than 2 weeks; storage for longer periods at 4°C or freezing of the DNA resulted in high backgrounds on genome-scanning Southern blots, presumably because of a reduction in size of the DNA. (Preparation of genomic DNA in agarose plugs, as in pulsed-field gel electrophoresis applications, may offer an acceptable alternative.) Digestion of genomic DNAs was performed with restriction endonucleases (Bethesda Research Labs., New England Biolabs), and the digested samples were separated by electrophoresis through either 0.6% or 0.8% agarose gels (SeaKem GTG, FMC, Rockland, ME) in TBE buffer [90 mM tris-borate and 1 mM EDTA (pH 8.3)]; the buffer was circulated (anode to cathode) at a rate of 250 ml/hour. The bed dimensions of the horizontal gel apparatus (Dan-Kar, Reading, MA) were 20 cm (width) by 60 cm (length). For some experiments, the gels were cast in wedge shape (by propping up the box at the cathode end before the gel was poured; for example, gel thickness at cathode end of 5 mm, gel thickness at anode end of 1 cm, buffer depth over cathode end of 4 mm, and buffer depth over anode end of 4 mm). Electrophoretic separation was at 185 V for 20 to 30 hours. The DNA in the gels was transferred to Gene Screen Plus membranes (Du Pont). The filters were placed in sealed plastic bags, incubated at room temperature with 4× SSPE [1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$ (pH 7.7), and 1 mM EDTA] for 30 min and then at 65°C in 0.5 M $NaPO_4$ (pH 7.1), 2 mM EDTA, 7% SDS, and 0.1% sodium pyrophosphate [G. M. Church and W. Gilbert, *Proc. Natl. Acad. Sci U.S.A.* **81**, 1991 (1984)] for 4 to 8 hours, and hybridized at 65°C in a fresh sample of the latter buffer containing 2×10^6 to 4×10^6 cpm of DNA probe per milliliter for 24 to 36 hours. The filters were then sequentially washed (twice; 20 min per wash) in 800 ml of the following: 2× SSC (standard saline citrate) at room temperature; 2× SSC containing 0.1% SDS at 65°C; 1× SSC containing 0.1% SDS at 65°C; 0.5× SSC containing 0.1% SDS at 65°C; and 0.2× SSC containing 0.1% SDS at 65°C. The filters were blotted dry, wrapped in plastic wrap, and exposed for 24 to 36 hours to XAR-5 film (Kodak) with a Lightning Plus intensifying screen (Du Pont) at −70°C.
22. A plasmid clone, pHR3.0, containing 3 kb of the rat tyrosine hydroxylase gene {previously mapped to the distal region of mouse chromosome 7 [M. H. Brilliant, M. M. Neimann, E. M. Eicher, *J. Neurogenet.* **4**, 259 (1987)]; provided by D. M. Chikaraishi and E. J. Lewis} was labeled with ^{32}P by nick-translation [P. W. J. Rigby, M. Diekman, C. Rhodes, P. J. Berg, *J. Mol. Biol.* **113**, 237 (1977)] to a specific activity of $>10^8$ cpm/μg. The 28RN probe (Fig. 2) was labeled with ^{32}P by the random primer method (*19*) to a specific activity of 1×10^8 to 5×10^8 cpm/μg.
23. We thank S. A. Deveau, B. K. Lee, and L. L. Washburn for technical assistance; and E. H. Birkenmeier, E. L. Kuff, K. K. Lueders, R. W. Melvold, B. Mintz, A. M. Skalka, J. C. Stone, and H. G. Wolfe for helpful discussions. Supported by NIH grants GM43840, CA06927, and RR05539, by an appropriation from the Commonwealth of Pennsylvania (The Institute for Cancer Research), by NIH grants CA34196, GM20919, and RR01183, and by American Cancer Society grant 1N155 (The Jackson Laboratory).

19 July 1990; accepted 11 February 1991

Determination of Primary Motoneuron Identity in Developing Zebrafish Embryos

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The developmental determination of primary motoneurons was investigated by transplanting identified motoneurons in embryonic zebrafish to new spinal cord positions. Some cells moved from the new positions in which they were placed back to their original positions, thus it was difficult to evaluate whether they were determined. Among cells that remained in their new positions, those transplanted about 1 hour before axogenesis developed axonal trajectories that were appropriate for their original soma positions, whereas those transplanted 2 to 3 hours before axogenesis developed morphologies appropriate for their new soma positions. These results suggest that motoneuronal identity is determined before axogenesis.

NERVOUS SYSTEMS CONSIST OF many different types of neurons that have characteristic shapes and patterns of synaptic connectivity. In some organisms, including many invertebrates (*1*) and the embryos of some fishes (*2*), certain neurons can be uniquely identified on the basis of morphological criteria such as soma position and axonal trajectory. When do the individual identities of these neurons become determined (*3*) so that their developmental potentials are restricted and are independent of their positions? To address this question, I transplanted identified motoneurons from their normal spinal cord positions in donor embryonic zebrafish to new spinal cord positions in host embryos (*4*). Transplanted motoneurons were considered undetermined if their axonal trajectories were appropriate for their new soma positions and determined if their trajectories were appropriate for their original soma positions. These studies suggest that the identities of individual primary motoneurons are determined before axogenesis.

The segmentally arranged axial muscles of the zebrafish trunk are innervated by a segmentally repeated set of three or four primary motoneurons (*5, 6*). Even before axogenesis, each motoneuron within this set is individually identifiable by the axial position of its soma within the spinal cord. Moreover, the growth cone of each motoneuron selects a different pathway as it extends directly to the muscle territory appropriate for its adult function (Fig. 1). Thus, within each set of motoneurons, each cell develops a unique morphology.

Previous studies have shown that individual primary motoneurons can be precisely identified and ablated with laser irradiation before axogenesis (*5, 7, 8*). To verify that individual primary motoneurons could be identified for transplantation with the same precision, I removed one or more specific primary motoneurons from trunk segments 6 to 10 of 16- to 18-h (hours after fertilization at 28.5°C) embryos. In trunk segments, CaP and MiP primary motoneurons typically undergo axogenesis around 17 h and 18 to 19 h, respectively; therefore, these experiments were performed ~1 hour before axogenesis. Embryos were allowed to develop until 25 h and were then fixed, and the remaining primary motoneurons were labeled with the zn-1 monoclonal antibody (*7*). In every case, the primary motoneurons that had been removed were absent (Fig. 1) (*9*). In all but one instance, all of the other primary motoneurons in the spinal segment were present at 25 h.

To investigate whether the individual identities of primary motoneurons were determined before axogenesis, I transplanted identified motoneurons isochronically from the CaP and MiP positions ~1 hour before axogenesis (Figs. 1 and 2) (*10*). No attempt

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was made to transplant cells to host segments corresponding to the segment of origin (*11*), although no obvious differences were observed between motoneurons that were moved to positions corresponding to their original segments and those moved to new segments. This finding is consistent with observations from avian and amphibian embryos that show that motoneurons do not distinguish between muscles derived from correct and incorrect segments (*12*).

All of the surviving transplanted cells (26%) extended growth cones along pathways that were normal for motoneurons as a class. Thus, by the time these cells were transplanted, they were probably already determined to be motoneurons and could not, for example, develop into VeLD interneurons (*13*), despite the similar positions of the MiP and VeLD somata (Fig. 1). To investigate whether the individual identities of these primary motoneurons were also determined by this stage, I examined their soma positions and axonal trajectories.

Table 1. Soma position and morphology of transplanted motoneurons.

Time before axogenesis (hours)	*n*	Original soma position	New placement of soma	Final soma position	Axonal trajectory
1	4	CaP	CaP	CaP*	CaP
1	3	MiP	MiP	MiP*	MiP
1	1	CaP	MiP	MiP†	CaP
1	3	MiP	CaP or RoP	CaP or RoP†	MiP
1	4	CaP	MiP	CaP‡	CaP
1	2	MiP	CaP	MiP‡	MiP
2	3	CaP	CaP	CaP*	CaP
2 to 3	3	MiP	MiP	MiP*	MiP
2	2	CaP	MiP	MiP§	MiP
2 to 3	6	MiP	CaP or RoP	CaP or RoP§	CaP or RoP
2	6	CaP	MiP	CaP‡	CaP
2 to 3	1	MiP	CaP	MiP‡	MiP

*Controls. †Cells determined at the time of transplantation. ‡Somata moved back to their original positions. §Cells undetermined at the time of transplantation.

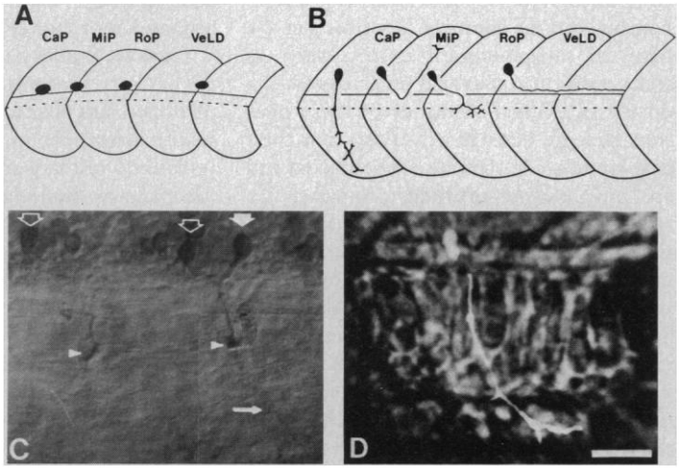

Fig. 1. Morphology and positions of primary motoneurons and interneurons. (**A** and **B**) Each spinal segment contains three primary motoneurons—CaP, MiP, and RoP—and one primary interneuron, VeLD (*13*), all of which can be identified by their individual morphologies. Several consecutive muscle segments are shown; for simplicity, a single primary motoneuron or primary interneuron is shown in each segment. The names of the neurons are given at the top of each segment. (A) shows the positions of these neurons before axogenesis in a 16-h embryo and (B) shows their morphologies at 24 h. In this and all subsequent figures dorsal is to the top and rostral is to the left. The solid horizontal line represents the ventral boundary of the spinal cord, and the dashed line represents the horizontal septum, which is the division between dorsal and ventral muscle. (**C**) Single motoneurons can be removed by aspiration. A cell was aspirated from the CaP position of the rostral segment at 16 h. At 25 h, the embryo was fixed and processed for zn-1 immunoreactivity (*7*). The CaP soma (solid vertical arrow) is present in the adjacent segment, and the CaP axon (solid horizontal arrow) extends into the ventral muscle. The CaP soma is absent from the segment from which it was aspirated. The MiP soma (open arrow) is present in both segments. In the segment without the CaP neuron, the MiP growth cone stopped at the horizontal septum (arrowhead) in a normal manner. (**D**) Motoneurons transplanted before axogenesis develop recognizable primary motoneuronal morphologies. A single motoneuron was transplanted from the CaP position on the right side of segment 10 of a 15.5-h labeled donor to the CaP position on the right side of segment 8 of a 15.5-h unlabeled host. This image-processed photomicrograph shows the cell at 23 h; the cell developed a normal CaP morphology. Bar represents 40 μm (A and B) or 25 μm (C and D).

The behavior of the transplanted cells fell into several different categories (Table 1). Cells that were transplanted from the CaP position to the CaP position or from the MiP position to the MiP position developed normal morphologies appropriate for their soma positions (Fig. 1). However, cells that were transplanted from the CaP position to the MiP position or from the MiP position to the CaP position behaved in two different ways. Several of these motoneurons developed morphologies in which their axonal trajectories were appropriate for their original soma positions, and not for their new soma positions (Fig. 2). For example, one cell that was transplanted from the CaP position to the MiP position extended its axon along the CaP-specific pathway into the ventral muscle, and three cells transplanted from the MiP position to the CaP or RoP position extended axons along the normal MiP-specific pathway into the dorsal muscle. Thus, the identity of these cells appears to have been determined because they developed axonal trajectories appropriate for their original soma positions and not for their new soma positions; their axonal trajectories were therefore independent of their soma positions.

The remaining motoneurons also developed axonal trajectories that were appropriate for their original soma positions. However, there was a key difference in their development from that of the cells just described. Surprisingly, by 2 hours after transplantation, the somata of this second group of cells had moved from the new positions in which they were placed to positions that corresponded to their original positions. For example, the somata of four cells that were transplanted from the CaP position to the MiP position moved back to the CaP position (Fig. 2C, inset), and the somata of two cells transplanted from the MiP position to the CaP position moved back to the MiP position. All of these cells extended axons along the pathways appropriate for their original soma positions (Fig. 2). However, because the somata of these cells did not stay in the new positions in which they were placed, these axonal trajectories were also appropriate for their new soma positions. Thus, it was not possible to conclude that the identity of these cells was determined because it was not clear whether their axonal trajectories were independent of their soma positions.

To investigate whether the identities of younger motoneurons were determined, I performed similar transplants at earlier developmental stages (Table 1). Cells were transplanted from the CaP position at 15 to 15.5 h (~2 hours before they would normally undergo axogenesis) and from the MiP position at 16 to 16.5 h (~2 to 3 hours

before they would normally undergo axogenesis). As in the previous experiments, cells that were transplanted from the CaP position to the CaP position or from the MiP position to the MiP position developed normal morphologies appropriate for their soma positions. Cells that were transplanted from the CaP position to the MiP position or from the MiP position to the CaP or RoP position again behaved in two different ways. Some of these cells developed axonal trajectories that were appropriate for their new soma positions. For example, two of the cells that were transplanted from the CaP position to the MiP position developed normal MiP morphologies, and six of the cells transplanted from the MiP position to the CaP or RoP position developed normal CaP or RoP morphologies, respectively (Fig. 3). Because these cells developed in ways appropriate to their new soma positions, their identities appear to have been undetermined. The remaining motoneurons developed axonal trajectories that were appropriate for their original soma positions. However, as with the cells that were transplanted at later developmental stages, the somata of these remaining cells moved from the new positions in which they were placed back to their original positions, making it difficult to evaluate whether their identities were determined.

My results suggest a number of features about the process by which the identities of individual primary motoneurons become determined. A surprising finding was that after they were transplanted to new positions, the somata of many primary motoneurons moved back to their original positions. Similar results have been described for gastrula cells in avian embryos (*14*), and these findings are consistent with the idea that cells may recognize local positional cues. Although the ability of these cells to move after transplantation made it difficult to evaluate whether they were determined, this ability might be a reflection of the cells having undergone an early step in their determination that occurs before determination of axonal trajectory. The fact that these cells appeared to be determined as primary motoneurons before they were determined for development of particular axonal trajectories is consistent with the idea that determination of primary motoneuronal identity may be a multistep process. It can be concluded from the instances in which transplanted primary motoneurons remained where they were placed that these cells were undetermined 2 to 3 hours before axogenesis but that their individual identities were determined by 1 hour later. These results suggest that, initially, primary motoneurons from both the CaP and MiP positions may constitute an equivalence group (*15*); however, the results do not rule out other possibilities.

Before these cells are determined, the position of the soma of a primary motoneuron appears to influence its axonal trajectory, implying that local cues in the vicinity of the soma regulate axonal morphology. Two neighboring cell types, primary motoneurons and floor plate cells, cannot be sole providers of such cues because removal of one or two identified primary motoneurons by laser irradiation or the absence of floor plate cells in *cyc-1* mutants (*16*) does not alter primary motoneuronal morphology (*17*). However, the putative positional cues could be provided by a combination of sources, including motoneuronal somata and the floor plate. Although they were often placed in new orientations, transplanted cells sprouted growth cones with the correct polarities (*18*) (Fig. 1). Thus, either the cells rotated to reestablish their original polarities, or they established new polarities appropriate for their new positions. In either case, this result suggests that in addition to local positional cues there may also be local polarity cues. Learning the nature and distribution of these putative cues should provide insight into the mechanisms involved in determination of motoneuronal identity.

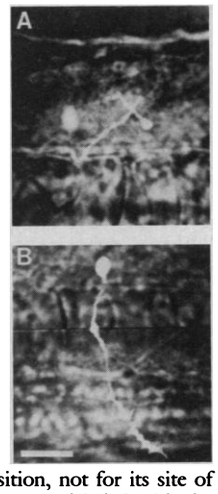

Fig. 3. Motoneuronal axon trajectories can be influenced by soma position. Some motoneurons transplanted 2 to 3 h before axogenesis developed morphologies appropriate for their new soma positions. (**A**) The labeled cell was transplanted from the CaP position of a 15.5-h donor to the MiP position of a 15-h host. This image-processed photomicrograph shows the cell at 26 h; the cell developed a normal MiP morphology appropriate for its new soma position, not for its site of origin. The donor embryo was labeled with the zn-1 monoclonal antibody at 25 h to verify that CaP was absent and that MiP and RoP were present in the donor segment. (**B**) The labeled cell was transplanted from the MiP position of a 16.5-h donor to the CaP position of a 16-h host. This image-processed photomicrograph was taken at 26 h; the cell developed a normal CaP morphology appropriate for its new soma position, not for its site of origin. Of the five instances in which cells transplanted from the MiP position developed as CaPs, two of the donor embryos were examined at 25 h to verify that MiP was absent and that CaP and RoP were present in the donor segment. Bar represents 25 μm.

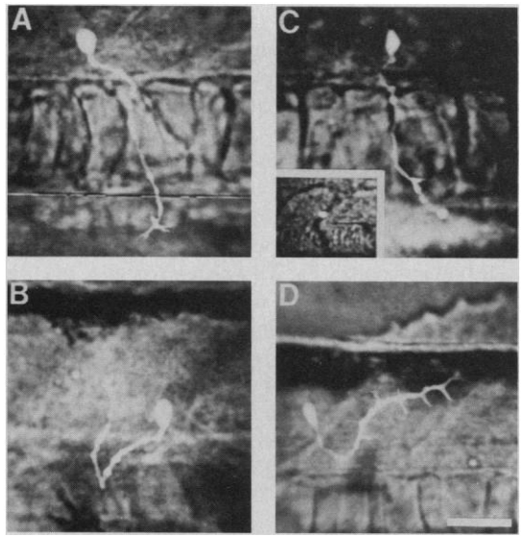

Fig. 2. The identities of individual primary motoneurons are determined before axogenesis. Motoneurons were transplanted from the CaP or MiP position of trunk segments 7 to 11 of 16- to 18-h–old rhodamine-dextran–labeled donor embryos to unlabeled host embryos ~1 hour before axogenesis. All of these cells extended axons along pathways appropriate for the original spinal cord positions of their somata. (**A**) The labeled cell was transplanted from the CaP position of a 16-h donor to the MiP position of a 16-h host. The axonal trajectory of this cell, shown here at 25 h did not correlate with the soma position; although the soma remained in the MiP position, the axon extended along the CaP pathway. (**B**) The labeled cell was transplanted from the MiP position of a 17-h donor to the RoP position of a 17-h host. The soma position and axonal trajectory of this cell, shown here at 26 h, did not correlate; the soma remained in the RoP position, but the axon extended along the MiP pathway of the next rostral segment. (**C**) The labeled cell was transplanted from the CaP position of a 16.5-h donor to the MiP position of a 16-h host. The soma moved back to the CaP position, however, and the cell developed a normal CaP morphology, shown here at 23 h. (**Inset**) The soma was present in the MiP position 10 min after the transplant. (**D**) The labeled cell was transplanted from the MiP position of a 17-h donor to the CaP position of a 17-h host. The soma moved back to the MiP position and the cell developed a normal MiP morphology, shown here at 24 h. Bar represents 25 μm (A to D) or 40 μm (C, inset).

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4. Embryos of the zebrafish, *Brachydanio rerio*, were obtained from our laboratory colony and maintained as described (*18*). The developmental stage of embryos was evaluated by the number of somite pairs; there are six pairs at 12 h and two pairs are added per hour until the embryo has 30 pairs [E. Hanneman, B. Trevarrow, W. K. Metcalfe, C. B. Kimmel, M. Westerfield, *Development* **103**, 49 (1988)]. Donor embryos were labeled at the 1- to 16-cell stage by injecting the yolk cell with 5 to 10% rhodamine-dextran as described [R. K. Ho and D. Kane, *Nature* **348**, 728 (1990)]. The label rapidly diffused into all of the blastomeres by way of cytoplasmic bridges. Embryos developed until primary motoneurons could be identified by morphological criteria (*5, 7*). One or two identified motoneurons were removed from the donor embryo by aspiration with a micropipette that was prepared on a Flaming-Brown P80/PC micropipette puller (Sutler Instrument Co.); tips were broken manually to a diameter of 8 to 12 μm. Micropipettes were backfilled with light mineral oil, and connected to a 10-μl Hamilton syringe by means of mineral oil–filled polyethylene tubing. A labeled donor embryo and an unlabeled host embryo were mounted side by side in agar (*7*). The labeled cells were expelled into the host embryo by gentle pressure. Only a single, large cell was placed in any one spinal segment. For all of the experiments described in this paper, the native primary motoneurons were eliminated from recipient spinal segments by aspiration before the transplant, although their elimination was not verified in every case. The development of transplanted cells was followed with a low-light level video system. Bright-field and fluorescence images were stored separately on optical disk. Images were processed to enhance contrast and to add multiple focal planes, bright-field, and fluorescence images on a MacIntosh computer with NeuroVideo and Image software.
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9. In ten trials, one or two neurons were removed from embryos ranging in age from 16 to 18 h. In nine of these trials, each of the four motoneurons—CaP, VaP, MiP, and RoP—was positively identified, whether removed or remaining. In one trial, three cells were positively identified—including the removed MiP—and one cell, RoP, remained unaccounted for.
10. Single cells were taken from the CaP position of segments in which a VaP was not present, or a pair of cells were taken from the CaP and VaP positions of the same donor segment—at this early stage, it is not possible to distinguish CaP from VaP (*5*). Cells from both the CaP and VaP positions of a single segment can develop as normal CaPs when transplanted to a host in which the native primary motoneurons have been removed (*17*). These cells thus appear to have equivalent developmental potentials.
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6 August 1990; accepted 22 January 1991

OPC-21268, An Orally Effective, Nonpeptide Vasopressin V1 Receptor Antagonist

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An orally effective, nonpeptide, vasopressin V1 receptor antagonist, OPC-21268, has been identified. This compound selectively antagonized binding to the V1 subtype of the vasopressin receptor in a competitive manner. In vivo, the compound acted as a specific antagonist of arginine vasopressin (AVP)–induced vasoconstriction. After oral administration in conscious rats, the compound also antagonized pressor responses to AVP. OPC-21268 can be used to study the physiological role of AVP and may be therapeutically useful in the treatment of hypertension and congestive heart failure.

TWO SUBTYPES OF PERIPHERAL AVP receptors have been distinguished both functionally and pharmacologically. In kidney, AVP exerts an antidiuretic effect through V2 vasporessin receptors by an adenosine 3′,5′-monophosphate (cAMP)–dependent mechanism (*1*). In liver and vascular smooth muscle, AVP elicits glycogenolysis and vasoconstriction, respectively, through V1 receptors by a cAMP-independent mechanism that is coupled to phosphoinositide turnover (*2*). Although many vasopressin antagonists have been developed for therapeutic use, these antagonists are all peptide analogs (*3*) and therefore do not have high enough oral bioavailability. We now describe a nonpeptide V1 vasopressin receptor antagonist, OPC-21268 (1-{1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone) (Fig. 1), which was developed by optimization of the lead molecule found from random screening of several thousands of compounds.

AVP and OPC-21268 both displace ^{3}H-labeled AVP bound to rat liver (V1 receptor) and kidney (V2 receptor) plasma membranes (Fig. 2). OPC-21268, at concentrations of 10^{-7} to 10^{-5} M, caused a concentration-dependent displacement of [^{3}H]AVP binding to V1 receptors, but the inhibitory effect of OPC-21268 on V2 receptor binding was weak. The concentration of OPC-21268 that displaced 50% of specific AVP binding (IC_{50}) was 4×10^{-7} M for V1 receptors and $>10^{-4}$ M for V2 receptors. From the IC_{50}, we calculated the inhibition constant (K_i) of OPC-21268 for V1 receptors (1.4×10^{-7} M). To determine if OPC-21268 interacts competitively or noncompetitively with V1 receptors, we analyzed [^{3}H]AVP binding data from rat liver membranes in the presence and absence of OPC-21268 by the Lineweaver-Burk method. OPC-21268 reduced the slope but did not change the y-axis intercept (Fig. 2B). These data indicate that OPC-21268 inhibited [^{3}H]AVP binding by changing the dissociation constant (K_d) but without changing the maximum number of receptors (B_{max}). Thus, OPC-21268 interacts competitively with V1 receptors. The K_d (1×10^{-7} M) of OPC-21268 for V1 receptors was consistent with the K_i obtained from displacement experiments. These data suggest that OPC-21268 selectively antagonized V1 receptors in vitro.

To study whether OPC-21268 acts as a specific antagonist of V1 receptors in vivo, we examined the effects of intravenous OPC-21268 administration in pithed rats

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Fig. 1. Structure of OPC-21268, 1-{1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1*H*)-quinolinone.