



US troops under threat of chemical warfare don gas masks and chemical suits during the 1991 Gulf War.

leagues⁴ when they discovered a threefold increase in the frequency of central nervous system symptoms (headaches, insomnia, drowsiness, nervousness, unfocused attention, and impaired capacity to conduct simple calculations) following pyridostigmine ingestion by 213 Israeli soldiers treated during the Persian Gulf War! A report in this issue of *Nature Medicine* by Friedman *et al.*⁵ follows up on this intriguing observation. The authors used a simulated stress situation in mice (the forced-swim protocol) to explore whether the phenomenon noted in Israeli troops might be related to increased penetration of pyridostigmine into the brain under conditions of increased stress (such as occur in war). They found that in stressed mice the peripherally administered dose of pyridostigmine necessary to inhibit brain acetylcholinesterase by 50% was 1/100th that required in nonstressed mice. Brain levels of *c-fos* oncogene and of acetylcholinesterase mRNA were also increased under the stress conditions. (There is a *c-fos* binding site in the mammalian acetylcholinesterase promoter). In addition, Friedman and colleagues analyzed pyridostigmine's effects on 35 normal, healthy volunteers during peacetime in a double-blind study. They report that in the peacetime group documented symptoms were restricted to perturbations of the peripheral nervous system (diarrhea, excess sweating, increased salivation). By contrast, predominantly central nervous system symptoms were reported by the Israeli soldiers receiving pyridostigmine pretreatment during wartime. These studies demonstrate a significant correlation between stress and pyridostigmine-induced effects in the central nervous system suggesting that, in troops exposed to emotional stress under conditions of war, the BBB may have unexpectedly become more permeable to administered pyridostigmine.

The concept of stress-induced alterations in BBB permeability has been studied previously in several different animal models. Acute immobilization stress in rats^{6,7}, cold or isolation exposure in mice⁸, and exposure of rats to conditions of acute as well as chronic summer heat⁹, all resulted in increased penetration of the BBB by drugs, neurotransmitters and viruses that are normally excluded. Conceivably, the increased BBB penetration by pyridostigmine in soldiers serving in the Gulf War may have been further exacerbated by the hot weather conditions in that geographic area.

Irrespective of what may have been the cause of this phenomenon, the paper by Friedman and co-workers brings to light an important phenomenon which, to date, may not have been given the attention that it deserves. Specifically, their findings and those of other reports indicate that we must look at the BBB from a different perspective than the classical textbook view, which teaches that the BBB is a very effective barrier to penetration of undesirable molecules into the brain. This may certainly be the case under normal conditions but during stress, this premise appears to fall apart. A better understanding and awareness of this important phenomenon is essential for designing appropriate pharmacotherapy for use during war and peace.

1. Rowland, L.P., Fink, M.E. & Rubin, L. Cerebrospinal fluid: Blood-brain barrier, brain edema and hydrocephalus. in *Principles of Neural Science* (eds. Kandel, E.R., Schwartz, J.H. & Jessell, T.M.) 1050–1060 (Elsevier, New York, 1991).
2. Deyi, X., Limxiu, W. & Shuqi, P. The inhibition and protection of cholinesterase by physostigmine and pyridostigmine against soman poisoning *in vivo*. *Fundam. Appl. Toxicol.* **1**, 217–221 (1981).
3. Diruhumber, P., French, M.C., Green, D.M., Leadbeater, L. & Stratton, J.A. The protection of primates against soman poisoning by pretreatment with pyridostigmine. *J. Pharmacol.* **31**, 295–299 (1979).
4. Sharabi, Y. *et al.* Survey of symptoms following intake of pyridostigmine during the Persian Gulf War. *Isr. J. Med. Sci.* **27**, 656–658 (1991).
5. Friedman, A. *et al.* Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. *Nature Med.* **2**, 1382–1385 (1996).
6. Belova, I. & Jonsson, G. Blood-brain barrier permeability and immobilization stress. *Acta Physiol. Scand.* **116**, 21–29 (1982).
7. Dvorska, I. *et al.* On the blood-brain barrier to peptides: Effects of immobilization stress on regional blood supply and accumulation of labelled peptides in the rat brain. *Endocr. Res.* **26**, 77–82 (1992).
8. Ben-Nathan, D., Lustig, S. & Danenberg, H.D. Stress-induced neuroinvasiveness of a neurovirulent noninvasive Sindbis virus in cold or isolation subjected mice. *Life Sci.* **48**, 1493–1500 (1991).
9. Sharma, H.S., Nyberg, F., Cervos-Navarro, J. & Dey, P.K. Histamine modulates heat stress-induced changes in blood-brain barrier permeability, cerebral blood flow, brain oedema and serotonin levels: an experimental study in the young rat. *Neuroscience* **50**, 445–454 (1992).

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Hedgehogs in the clinic

Components of the *hedgehog* signaling pathways play key roles during embryonic development and in several human diseases.

The importance of cell-cell interactions in regulating vertebrate development has been understood for many years. However, it is only relatively recently that the principal players have been identified. Prominent among these is the *hedgehog* (HH) family of signaling peptides first identified in pioneering genetic screens in the fruit fly, *Drosophila*¹.

Mammals have three hedgehog genes, *Sonic (Shh)*, *Indian (Ihh)* and *Desert (Dhh)* *Hedgehog*². For the most part these are expressed in non-overlapping patterns in a wide variety of tissues. Thus, the expectation has been that HH signaling is likely to play an important role in many distinct developmental processes. This expectation is borne out by recent reports from a num-

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ber of groups^{3–5}. Moreover, there is the first, and probably not the last, direct association of

a HH mutant, *SHH*, with a particular clinical disease in humans^{6,7}. Finally, genetic and biochemical dissection of HH signaling is starting to shed light on its unexpected relationship with cholesterol metabolism and cancer^{8–13}.

Of the three mammalian HHs, *Sonic hedgehog* has occupied center stage. In the early mouse embryo and equivalent stages for all other vertebrates thus far examined, *Shh* is expressed in a major organizing center, the notochord, a rod of mesoderm that lies underneath the neural plate and extends along most the length of the body axis¹⁴. Removal of the embryonic notochord leads not only to the loss of ventral struc-

tures in the overlying central nervous system (CNS), but also to loss of the axial skeleton and muscles, both of which arise from paired blocks of paraxial mesoderm, the somites, lying on either side of the neural tube¹⁵. These studies, and a variety of *in vitro* reconstitution experiments, indicate that notochordally derived signals are responsible for short- and long-range induction of a variety of distinct derivatives within the CNS and somites¹⁴.

SHH is both necessary and sufficient for the induction of floorplate and motor neurons in spinal cord explants *in vitro*¹⁴. Like the notochord, the floorplate expresses *Shh*, and is thought, together with the notochord, to participate in the induction of adjacent ventro-lateral areas of the CNS, inducing motor neurons in the spinal cord, dopaminergic neurons in the mid-brain and cholinergic neurons in the fore-brain. As well as inducing motor neuron activity, *Shh* mimics the activity of the floorplate in dopaminergic¹⁴ and cholinergic neuron¹⁴ inductions. SHH is also able to induce vertebral precursors (sclerotome)¹⁴ and together with another family of signals, the Wnts, contributes to the development of muscle precursors (myotome)¹⁴ in presomitic mesoderm. Thus, patterning of a major part of the vertebrate body plan by these two organizing centers, the notochord and floorplate, is mediated by *Shh* signaling.

Further support for this argument comes from a recent paper in *Nature* describing *Shh* mutants generated by gene targeting in mice⁵. Such mutants, which almost certainly lack any signaling activity, survive to term, but not surprisingly, die shortly thereafter. The most distinctive superficial feature is absence of nasal structures and cyclopia resulting from the loss of ventral midline structures in the brain. In the spinal cord, despite the presence of the notochord, no floorplate or motor neuron induction is observed. These results confirm that *Shh* signaling plays a major role in organizing the ventral midline along the length of the CNS, from forebrain to spinal cord, although it is unclear to what extent different classes of ventral neurons are affected in the brain. It is surprising that in the somites, myotomal and sclerotomal cell types are not totally absent, only reduced, indicating the involvement of other midline signals in addition to *Shh*.

The head phenotype observed in *Shh* mutant mice is reminiscent of a human condition, holoprosencephaly (HPE), which is seen in 1 in 16,000 live births and

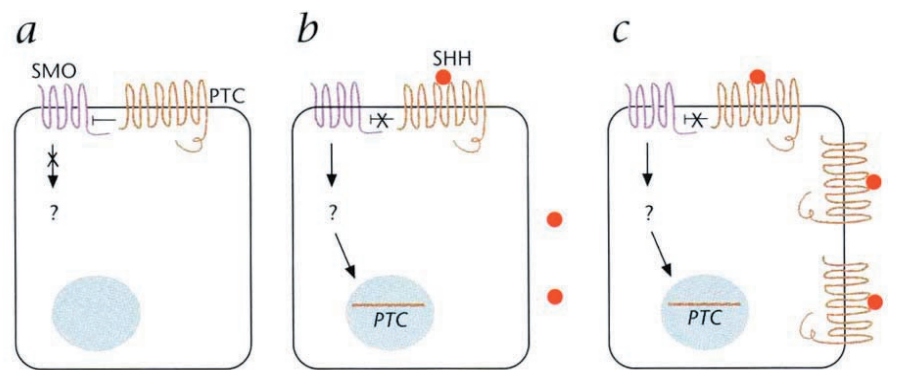


Fig. 1 A model of HH signaling. Schematic diagram of a model based on several lines of evidence to explain how the HH signal (SHH is used as an example) is transduced and modulated. In the absence of the HH signal (a), the constitutive activity of SMO is inhibited by PTC. Upon binding of SHH to PTC (b), the repression is relieved and the HH signal is transduced through SMO to activate downstream targets. One of the targets of the HH signal is *ptc*. Induction of *ptc* by HH may serve as a sink to terminate the HH signal to restrict its further diffusion to adjacent responsive cells (c). (Arrows indicate activation and bars indicate repression).

1 in 250 induced abortions¹⁶. HPE is also a common outcome of teratogenic studies using inhibitors of cholesterol metabolism¹⁷. Is there a connection?

Mapping of a number of families with rare autosomal dominant forms of this disorder has identified a number of candidate loci. Belloni *et al.*, reporting in the November issue of *Nature Genetics*⁶, have now mapped human *SHH* to chromosome 7q36, placing it close to one of the candidate loci, HPE3 (ref. 18). Indeed, they show that *SHH* maps within 15–250 kilobases of chromosomal rearrangements associated with HPE, suggesting a long-range influence on the regulation of *SHH* expression⁶. In an accompanying paper, Roessler and co-workers demonstrate a clear role for *SHH* in HPE by identifying a number of point mutations within the *SHH* gene associated with autosomal dominant familial HPE (ref. 7). However, in the mouse mutant, no dominant phenotype has been reported. Why then should there be a dominant phenotype in the human population? There are several simple explanations. One is the possible existence of modifiers in the genetic background that might influence the phenotype. This could be addressed in the mouse studies by out-crossing the *Shh* mutation onto random-bred backgrounds. Alternatively, the human *SHH* alleles might act as antimorphs, actually interfering with signaling by the wild-type protein. As alleles similar to those described in the human studies behave as true nulls in the fly, this would seem to be unlikely¹⁹. Finally, it may simply reflect

different dosage requirements for different species.

With respect to the occurrence of HPE following treatment with inhibitors of cholesterol biosynthesis, there may be a connection relating to the unusual biochemistry of SHH processing⁸. SHH is produced as a 46-kDa proform, which undergoes an autocatalytic cleavage to generate a 19-kDa signaling peptide¹⁴. Processing results in the covalent linkage of a single molecule of cholesterol at the carboxy terminal end of this peptide. As a result the peptide is associated with the cell surface. This is likely to be a critical factor because SHH has been implicated in the dose-dependent induction of different cell types^{14,20}. Consequently, inhibition of cholesterol biosynthesis might affect the processing and/or localization of SHH, thereby interfering with *Shh* signaling resulting in HPE.

In addition to HPE, a human congenital polysyndactyly has also been mapped to 7q36 (ref. 21). *Shh* signaling in the posterior mesenchyme of the vertebrate limb is associated with patterning of the digits, and through a complex feedback loop, with outgrowth of the limb bud²². Analysis of *Shh* mutants in the mouse clearly demonstrates that *Shh* is required for extension of the limb⁵. With the possibility of long-range interactions affecting *SHH* in HPE, it remains a possibility that similar long-range influences on *SHH* expression might underlie at least some forms of human polysyndactyly. In addition, the expression of *Shh* in many other structures at critical periods in their de-

velopment suggests that *Shh* signaling may be a key regulator of organogenesis in organs as diverse as the lung, tooth and hair²³. Further studies in the mouse, and increased clinical awareness in humans, will most likely produce evidence in support of this prediction.

What of *Dhh* and *Ihh*? Analysis of *Dhh* null mutants in the mouse³ indicates that *Dhh* is not required in the female. In contrast, males without *Dhh* are perfectly viable but infertile. *Dhh* is expressed in Sertoli cells, the principal somatic support cell for spermatogenesis, and in the absence of *Dhh* signaling, spermatogenesis fails. Thus, understanding the role of *Dhh* has clinical implications relating to infertility and contraception. In contrast, *Ihh* is expressed in cartilage and has been proposed to regulate the morphogenesis of skeletal elements through the regulation of a second secreted protein, parathyroid hormone-related peptide⁴. Further, the bone morphogenetic proteins, originally identified for their bone-inducing properties, are evolutionarily conserved targets of HH signaling¹⁴. Thus, it seems reasonable to expect that studying the action of *Ihh* is likely to shed light on cartilage and bone development.

Up until recently our understanding of how HH signaling is received and transduced came from genetic studies in the fly. These identified two cell-surface proteins, patched (PTC) and smoothed (SMO) that regulate HH signaling²⁴. Consequently, both are plausible candidates for the HH receptor. Now a reasonable model (see figure) as to how HH signaling may work has been formulated by a synthesis of genetic experiments in the fly (reported in *Cell*¹¹), and of biochemical experiments with vertebrate PTC (ref. 12, 13) and SMO (ref. 12) (published in a pair of papers in *Nature*). SMO, which shares similarity with seven-transmembrane G-coupled receptors, is required to transduce HH signal. In all likelihood it is constitutively active but activity is normally blocked by PTC (ref. 25), a 12-transmembrane protein with a topology similar to that of ion channels or transporters. A low level of PTC keeps SMO switched off in the absence of an HH signal. And, as would be predicted, loss of PTC leads to HH-independent activation of HH targets. Upon binding of SHH (and presumably other HH members in mammals because there appears to be only a single *Ptc* gene) to vertebrate PTC, repression of SMO by PTC is relieved and a signal is transduced through SMO. In support of this model,

genetic studies in the fly indicate that Hh most likely interacts with Ptc but requires Smo for transduction of the signal^{11,26}. Moreover, the recent *Nature* papers report that SHH binds directly to PTC (ref. 12, 13), but not to SMO (ref. 12), and SMO and PTC form a heteromeric protein complex that retains SHH binding¹². Finally, the resulting induction of *Ptc* on receipt of a HH signal, most likely sequesters additional HH, serving as a sink to limit diffusion of the HH signal to adjacent responsive tissue¹¹.

It is intriguing that mutations in *PTC* have been found in the human basal cell nevus syndrome and basal cell carcinoma^{9,10}, consistent with the model that HH signaling is now rendered constitutively active. However, it remains to be seen whether SMO is sufficient to transduce the HH signal. In addition, although the expression pattern of *Ptc* and *Smo* overlaps, there are regions in the mammalian embryo where *Smo* is expressed in the absence of *Ptc* (ref. 12). Whether SMO is actively transducing a HH signal in these regions remains to be established. As yet, there is no direct evidence that PTC also binds IHH and DHH and that their signals are transduced in a similar fashion, nor is there any detailed understanding of the essential features of signaling downstream of SMO.

In summary, the study of how HHs function has provided yet another example of how basic studies on a variety of organisms may provide a fundamental understanding of biological processes of direct relevance to the clinic. Clearly, much remains to be explained. One can expect in the future to see continuing efforts to determine how multiple HH signals are transduced, how the specificity of the response is controlled in different cell populations and how signaling by HHs and other secreted peptides is integrated to induce diverse tissues during embryonic development. In addition, HH, PTC and SMO offer attractive targets for drug-based approaches aimed at manipulating the interactions between these signaling components.

1. Nusslein-Volhard, C. & Wieschaus, E. Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801 (1980).
2. Echelard, Y. *et al.* Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75**, 1417–1430 (1993).
3. Bitgood, M.J., Shen, L. & McMahon, A.P. Sertoli cell signaling by Desert hedgehog regulates the male germ line. *Curr. Biol.* **6**, 298–304 (1996).
4. Vortkamp, A. *et al.* Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related

protein. *Science* **273**, 613–622 (1996).

5. Chiang, C. *et al.* Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* **383**, 407–413 (1996).
6. Belloni, E. *et al.* Identification of *Sonic hedgehog* as a candidate gene responsible for holoprosencephaly. *Nature Genet.* **14**, 353–356 (1996).
7. Roessler, E. *et al.* Mutations in the human *Sonic Hedgehog* gene cause holoprosencephaly. *Nature Genet.* **14**, 357–360 (1996).
8. Porter, J.A., Young, K.E. & Beachy, P.A. Cholesterol modification of hedgehog signaling proteins in animal development. *Science* **274**, 255–259 (1996).
9. Johnson, R.L. *et al.* Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* **272**, 1668–1671 (1996).
10. Hahn, H. *et al.* Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome. *Cell* **85**, 841–851 (1996).
11. Chen, Y. & Struhl, G. Dual roles for patched in sequestering and transducing hedgehog. *Cell* **87**, 553–563 (1996).
12. Stone, D.M. *et al.* Vertebrate homologues of patched and smoothed compose a receptor for sonic hedgehog. *Nature* **384**, 129–154 (1996).
13. Marigo, V., Davey, R.A., Zuo, Y., Cunningham, J.M. & Tabin, C.J. Biochemical evidence that patched is the hedgehog receptor. *Nature* **384**, 176–179 (1996).
14. Hammerschmidt, M., Brook, A. & McMahon, A.P. The world according to hedgehog. *Trends Genet.* (in the press).
15. Rong, P.M., Teillet, M.A., Ziller, C. & Le Douarin, N.M. The neural tube/notochord complex is necessary for vertebral but not limb and body wall striated muscle differentiation. *Development* **115**, 657–672 (1992).
16. Cohen, M.M. Perspectives on holoprosencephaly: Part I. Epidemiology, genetics, and syndromology. *Teratology* **40**, 211–235 (1989).
17. Roux, C. *Arch. Fr. Pediatr.* **21**, 451 (1964).
18. Marigo, V. *et al.* Cloning, expression, and chromosomal location of SHH and IHH: Two human homologues of the *Drosophila* segment polarity gene hedgehog. *Genomics* **28**, 44–51 (1995).
19. Porter, J.A. *et al.* The product of hedgehog autoproteolytic cleavage active in local and long-range signalling. *Nature* **374**, 363–366 (1995).
20. Ericson, J., Morton, S., Kawakami, A., Roelink, H. & Jessell, T.M. Two critical periods of sonic hedgehog signaling required for the specification of motor neuron identity. *Cell* **87**, 661–673 (1996).
21. Tsukurov, O. *et al.* A complex bilateral polysyndactyly disease locus maps to chromosome 7q36. *Nature Genet.* **6**, 282–286 (1994).
22. Maden, M. Developmental biology. The limb bud – Part two. *Nature* **371**, 560–561 (1994).
23. Bitgood, M.J. & McMahon, A.P. Hedgehog and Bmp genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. *Dev. Biol.* **172**, 126–138 (1995).
24. Perrimon, N. Serpentine proteins slither into the wingless and hedgehog fields. *Cell* **86**, 513–516 (1996).
25. Ingham, P.W. Localized hedgehog activity controls spatial limits of wingless transcription in the *Drosophila* embryo. *Nature* **366**, 560–562 (1993).
26. Alcedo, J., Ayzenzon, M., Von Ohlen, T., Noll, M. & Hooper, J.E. The *Drosophila* smoothed gene encodes a seven-pass membrane protein, a putative receptor for the hedgehog signal. *Cell* **86**, 221–232 (1996).

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