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Charles H. Turner · Alexander G. Robling

Mechanisms by which exercise improves bone strength

Abstract Certain exercises can induce osteogenesis and improve bone strength, yet the biological processes involved in bone mechanotransduction are only beginning to be understood. Several pathways are emerging from current research, including calcium signaling associated with membrane ion channels, adenosine triphosphate signaling, second messengers such as prostaglandins and nitric oxide, and signaling involving mitogen-activated protein kinase. One characteristic of the mechanosensing apparatus that has only recently been studied is the important role of desensitization. Experimental protocols that insert "rest" periods to reduce the effects of desensitization can double anabolic responses to mechanical loading. Exercises that reduce desensitization may provide an effective means to build bone strength.

Key words Bone mineral density · Calcium channel · ATP · Prostaglandins · Nitric oxide

Introduction

Mechanical stresses, and the resulting tissue deformation (strain), result from the loads carried by the skeleton. Stresses are not uniform throughout the bone but can be concentrated in certain regions, e.g., muscle attachments. Various exercises create different stress patterns in the skeleton. For instance, racquet sports selectively overload the dominant arm whereas sports that require jumping, such as volleyball, generate high stresses in the lower legs. The skeleton possesses an inherent biological control system that directs bone formation in response to high mechanical stresses (or strains), thus strengthening the skeleton in highly stressed regions. This system, sometimes called the "mechanostat" [1], involves the resident cells within bone tissue that detect and respond to mechanical loads. In regions of high stresses, bone formation is increased, particularly at periosteal bone surfaces, and bone turnover is reduced, reducing bone porosity. Consequently, mechanical loading can caused bones to increase their cross-sectional area and strengthen the bone tissue by decreasing porosity and consequently improving tissue density.

Efficiency of structures is enhanced if mass can be reduced as much as possible without compromising strength or rigidity. Hence, bone should not be formed in regions of low stress, as this adds little structural benefit and increases the risk of inappropriate bone formation that might impinge on nerves or other adjacent tissues, whereas adding bone to regions under high stress greatly improves bone strength. A recent study [2] shows how long bones apply this algorithm in response to loading. Cyclic mechanical loads were applied axially to the forelimbs of adult rats three times per week for 16 weeks. The rat ulna has a natural curvature in the mediolateral direction, so axial loads induce bending of the bone (Fig. 1). Under load, the medial periosteal surface of the bone was subject to compressive stresses and the lateral periosteal surface was under tension. The pattern of bone formation induced by loading resembles the strain distribution, with more bone formation where the strains are greatest. The improvement in bone structure is evidenced by a 69% increase in second moment of area (I). The ulnar bone strength in loaded limbs was 64% greater than control and energy absorbed before fracture increased by 94%, yet the improvement in areal bone mineral density (BMD) was only a modest 5%. Therefore, loading caused dramatic improvements in bone biomechanical properties, even with small changes in BMD. The structural efficiency of the ulna was improved by bone formation preferentially on the periosteal surface, with more bone formation in highly stressed areas where it was most needed.

C.H. Turner (✉) · A.G. Robling
Orthopaedic Research Laboratories and Biomechanics and Biomaterials Research Center, Indiana University Purdue University at Indianapolis, 541 Clinical Drive, Room 541, Indianapolis, IN 46202, USA
Tel. +1-317-274-3226; Fax +1-317-274-3702
e-mail: turnerch@iupui.edu

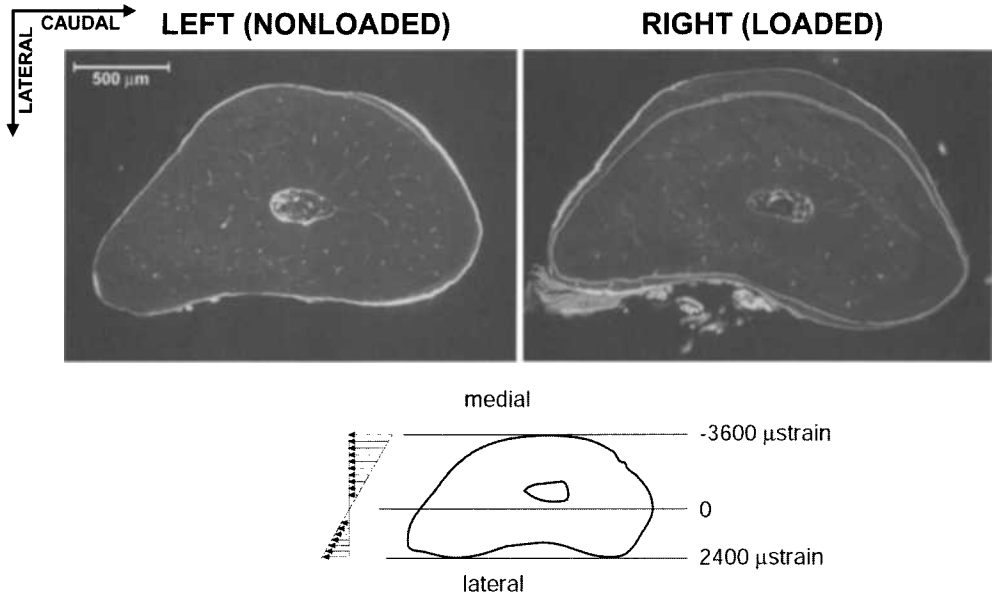

Fig. 1. Cross sections through the midshaft of the rat ulna. The rat ulna is strained more on the medial (*top*) surface when loaded. The *bottom figure* shows the strain profile across the loaded ulna. The strains are designated in units of microstrain (μstrain). Positive values are tensile strain and negative values are compressive strain. Bone formation is shown in the *right panel*. The *red lines* within the bone show labels at the beginning of loading. Little new bone was formed in the *left* (control) ulna, but the loaded ulna showed increased bone formation mostly in regions where the strains were of greatest magnitude. (Reprinted from Robling AG, Hinant FM, Burr DB, Turner CH. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. J Bone Miner Res 2002; 17:1545–1554. Used with permission from the American Society for Bone and Mineral Research)

Exercise as a therapy for osteoporosis

As illustrated in Fig. 1, mechanical loading can effectively strengthen bones and mechanical loading is an important regulator of skeletal development. However, bones become less sensitive to mechanical loading after skeletal maturity is reached (age 18–25 years). The addition of exercise to everyday activities provides a relatively ineffective treatment for adult osteoporosis [3]. A recent meta-analysis of controlled exercise trials likened exercise to calcium supplementation in its effect on bone, i.e., both cause a modest reduction of bone resorption, resulting in only 1%–2% gain in BMD per year [4]. It has proven to be very difficult to translate the bone-strengthening effects of mechanical loading seen in lab animals (Fig. 1) into effective exercise therapies for osteoporosis.

Osteogenesis is initiated only if mechanical loading is applied rapidly. Static loads do not induce osteogenesis in animal models whereas dynamic loading can be an effective stimulus for bone formation [5–7] (Fig. 2). In addition, animal studies have shown that the rate of loading or applied strain influences the osteogenic capacity of exercise [8]. Consequently, exercises that involve impact (such as jumping) are best for building bones [9]. Unfortunately, high-impact exercises are difficult for the frail and elderly and can aggravate osteoarthritis in the joints. Another challenge for anabolic exercise therapy is that bone tissue desensitizes to mechanical loading after a relatively few exercise repetitions. Perhaps the best example of mechanosensory desensitization in bone tissue is illustrated by two separate experiments in which animals were exposed to brief (1–50 cycles), moderate (50–100 cycles), or long (100–1600 cycles) periods of mechanical loading on a daily basis [10,11]. These experiments demonstrate that bone "tunes out" mechanical signals after a couple of dozen exercise cycles so that further exercise adds no further anabolic response (Fig. 3).

Strategies have been proposed to increase the anabolic effect of exercise by developing exercise regimens that are more effective and easier to accomplish. Studies in animals have shown that mechanical loading is much more anabolic if applied at a higher frequency. For instance, loading of rat forelimbs produced more than tenfold more bone formation when applied at 10 cycles/s (Hz), compared to application at 1 Hz [12]. In another study, small loads were applied to sheep hindlimbs at 30 Hz, 20 min per day for a year [13]. In this experiment, the loading was applied by a vibrating plate that the sheep stood on. Trabecular bone volume was increased by more than 30% in the femur (one of the bones subjected to loading), but areal BMD was not increased in the loaded bones, nor was cortical bone mass increased. The limited anabolic response in cortical bone to 30-Hz vibration could be due to the fact that peak strains in the bone were very small [about 5 microstrain (μstrain)]. Alternatively, it is possible that the bone tissue rapidly desensitized to the 30-Hz loading signal, thus limiting the anabolic response.

Recently, several studies have addressed the problem of bone cell desensitization to mechanical loading. The mechanisms of desensitization are much less well characterized for bone than for other systems, but certainly the desensitization pathways elucidated in other tissues can provide clues

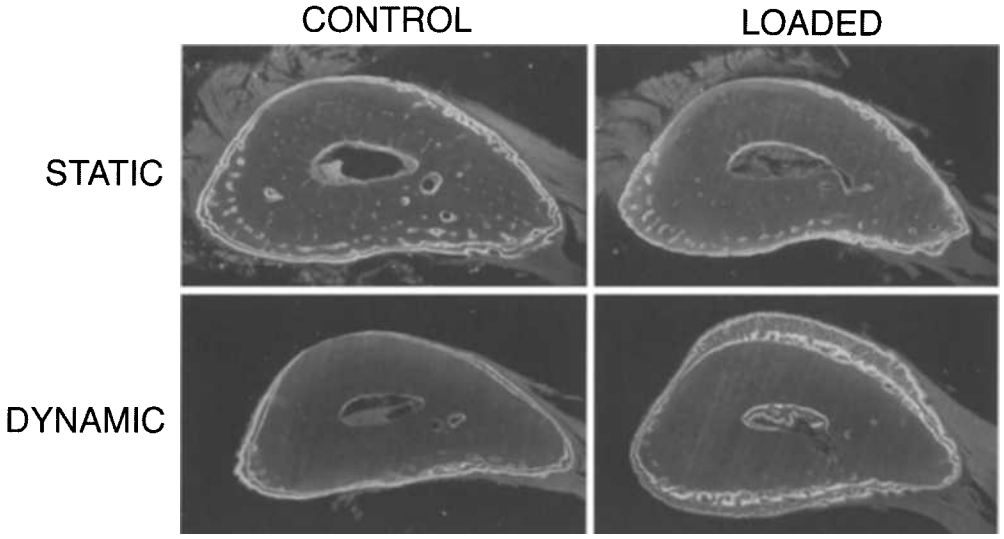

Fig. 2. Effect of static and dynamic loading applied to the rat ulna. Dynamic loading induced a potent osteogenic stimulus whereas there was a slight suppression of bone formation after exposure to static loading. The ulnar cross section in the *top right panel* received an axial load of 17 N for 10 min/day for 2 weeks, whereas the ulna in the *bottom right panel* received dynamic loading at 2 cycles per second for 10 min/day over the same study period. The *yellow lines* are calcein labels given 5 and 12 days after loading began. (Adapted from Robling AG, et al. Modulation of appositional and longitudinal bone growth in the rat ulna by applied static and dynamic force. Bone 2001;29:105–113)

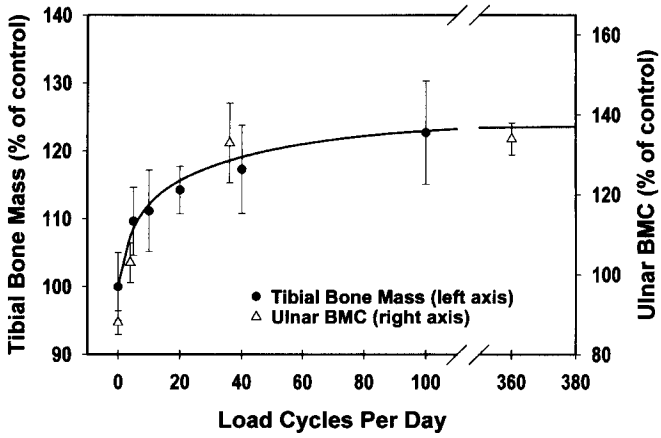

Fig. 3. Bone mass in the tibia of rats (*closed circles* [11]) or ulna of turkeys (*open triangles* [10]) increases after applied mechanical loading. However, the anabolic effect of loading saturates as the number of loading cycles increases. There is limited benefit of additional loading cycles above a couple dozen cycles per day. (From Burr DB, Robling AG, Turner CH. Effects of biomechanical stress on bones in animals. Bone 2002;30:781–786. Reprinted with permission from Elsevier)

for possible mechanisms in bone. For example, pressure sensitivity in skin baroreceptors (e.g., Pacinian corpuscle) involves a change in membrane potential (depolarization), but continued pressure by a constant-intensity stimulus quickly reduces the generator potential and returns the membrane potential to its resting state. Another possible pathway is G-protein-coupled receptor desensitization via receptor phosphorylation by several classes of kinases and eventually receptor sequestration/downregulation. Both these mechanisms could play a role in bone cell desensitization to mechanical stimulation: Ca^{2+} influx and G-protein signaling appear to be required for mechanotransduction to occur [14,15].

To illustrate mechanodesensitization in bone, we and others have performed a series of experiments showing that recovery periods, which allow the tissue to resensitize, enhance the effectiveness of mechanical loading on osteogenesis. Moreover, we have shown that there are different time scales, ranging from seconds to hours, required for resensitization to occur. In one experiment, rats were administered 36 loading cycles per day in a single bout, 5 days a week for 2 weeks. Some of the rats were given the 36 daily cycles consecutively, with no time between each cycle (back-to-back cycles), while other rats were given different durations of rest between each of their 36 daily cycles. Rats given 14 s between each load cycle formed 67% more bone on the endocortical surface than rats administered back-to-back cycles [16]. Similar experiments from other groups have confirmed the osteogenic benefit of short-term (on the order of seconds) recovery periods in restoring sensitivity to loaded bone [17,18]. At this point, the molecular mechanism of this desensitization remains unknown, but the fact that this phenomenon occurs on such a short time scale rules out several classical desensitization mechanisms, including receptor phosphorylation or internalization. A more likely candidate would involve electrophysiological effects within the cell (e.g., cytosolic versus endoplasmic reticulum Ca^{2+} levels) or membrane permeability to ions.

We have also observed resensitization of mechanically loaded bone that requires several hours. We subjected the right tibiae of rats to four separate bouts of loading per day for 3 nonconsecutive days [16]. Each bout comprised 90 load cycles; the only difference among the six groups of rats

was the amount of time allotted between each of the four daily loading bouts. The rats received 8, 4, 2, 1, 0.5, or 0 h between loading bouts, and their bone formation response was assessed 2 weeks later. The 8-h recovery group exhibited ~100% more bone formation that the 0- or 0.5-h recovery group, suggesting that the recovery periods restored a significant amount of mechanosensitivity to the bone; 4–8 h recovery appeared to fully restore mechanosensitivity. We repeated a portion of this experimental design in a long-term loading experiment using the rat ulna loading model developed previously by Torrance et al. [19]. Here we compared a standard loading protocol (360 consecutive cycles of loading) with a 3-h recovery protocol (four bouts of 90 cycles with 3 h between bouts) over a 16-week loading period, and found that both protocols significantly improved the bone strength in the loaded ulnae, but the ulnae from the recovery group exhibited 75% greater work to failure [20,21]. The loss and subsequent return of mechanosensitivity that occurs on the order of hours might be dependent on cell structural changes (e.g., architecture of the actin cytoskeleton) or on kinase-induced receptor phosphorylation and/or internalization. In vitro, reorganization of the osteoblast's actin cytoskeleton into stress fiber bundles is required for fluid shear-induced expression of genes linked to mechanotransduction and bone formation [22,23]. Reorganization of the actin cytoskeleton results in an increase in whole-cell stiffness, which might make detection of mechanical signals less effective. Alternatively, the signal transduction cascades activated by mechanical stimulation, which appear to involve autocraine/paracrine loops, involve several G-protein-coupled receptors (e.g., prostanoid receptors, adenosine triphosphate receptors) that might undergo uncoupling to their G-protein messengers by any of several kinases known to be expressed in bone cells.

The characteristics of the skeletal mechanotransducer

Mechanotransduction in bone involves several cell types. The cells that ultimately form or resorb bone may not be the same ones that transduce and respond to mechanical signals. Mechanotransduction might involve signaling through mechanically activated ion channels in the cell membrane, focal adhesions of the cytoskeleton, or a G-protein-coupled mechanoreceptor. In cultured osteoblastic cells, fluid flow increases intracellular calcium within minutes, and this response is suppressed by gadolinium, a blocker of the stretch-activated calcium channel [24] (Fig. 4). In addition, the L-type voltage-operated calcium channel probably plays a role in bone cell mechanotransduction. Studies using bone explants showed that gadolinium abolished loading-related responses in osteocytes, whereas a blocker of L-type calcium channels inhibited loading-related responses in osteoblasts [25]. In addition, two blockers of L-type calcium channels, verapamil and nifedipine, strongly suppress mechanically induced bone formation in rats [14,26].

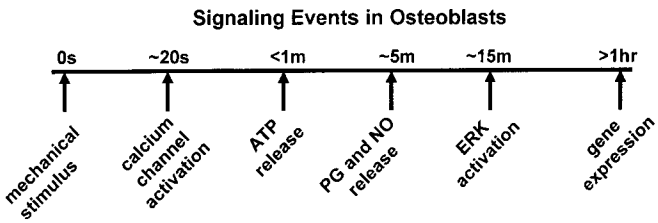

Fig. 4. Sequence of molecular events that occur after a mechanical stimulus and precede bone formation. *ATP*, adenosine triphosphate; *PG*, prostaglandin; NO, nitric oxide, *ERK*, extracellular signal-related kinase

An increase in intracellular calcium is observed in osteoblastic cells seconds after a mechanical stimulus. The combined effects of increased open frequency of membrane calcium channels (L-type voltage-operated and stretch-activated) and increased release of Ca^{2+} from intracellular stores mobilize intracellular calcium [22,27,28]. The inositol 1,4,5-triphosphate pathway plays a key role in intracellular calcium release after a mechanical stimulus [22,28], and intracellular calcium signaling appears to be requisite for expression of bone matrix proteins. Intracellular calcium mobilization triggers a mitogen-activated protein kinase (MAPK) signaling pathway, which is linked to the expression of osteopontin [28]. MAPK signaling involves the activation of extracellular signal-regulated kinase (ERK) within about 15 min after mechanical loading [29]. ERK activation might be initiated by prostaglandins or nitric oxide [30], which are released following mechanical loading. In addition, activation of focal adhesion kinase (FAK) may be required for ERK phosphorylation (activation) [31].

Osteoblastic cells attach to the bone matrix through the integrin–cytoskeleton complex. Integrins are heterodimeric transmembrane proteins that bind to the extracellular matrix (ECM) on the outside of cells and are linked to the actin cytoskeleton via the short cytoplasmic domain of the β-subunit on the inside of cells at specialized sites known as focal adhesions. Several lines of evidence obtained with various cell types, including fibroblasts, epithelial cells, endothelial cells, and neutrophils, as well as osteoblasts, indicate that a key molecule in mediating linkage of actin filaments to integrin cytoplasmic domains is the protein α-actinin [23,32]. Microinjection into osteoblasts of a dominant negative α-actinin causes the competitive displacement of the endogenous α-actinin from focal adhesions and blocks fluid flow-induced gene expression [23].

Autocrine and paracrine messengers for skeletal mechanotransduction

In cultured osteoblasts, fluid flow causes release of adenosine triphosphate (ATP), prostaglandins, and nitric oxide within minutes [33–37]. Mechanical stimuli also affect osteoclasts, but this effect appears to be indirect. When exposed to strain, marrow stromal cells (of osteoblastic lineage) reduce expression of osteoclast differentiation factor

(a.k.a. RANKL or TRANCE), which in turn decreases osteoclast number [38,39]. Consequently, cells of osteoblastic lineage appear to be mediators of the suppressive effects of mechanical stimuli on bone resorption.

Adenosine triphosphate (ATP) signaling plays a role in skeletal mechanotransduction. Osteoblastic cells can communicate through autocrine or paracrine activity of secreted ATP on $P2Y_2$ purinergic receptors [40], and $P2Y_2$ signaling appears to be mechanosensitive [33]. A local mechanical stimulus initiates intercellular calcium signaling, mediated by ATP receptors, which rapidly propagates from cell to cell. In addition, the P2X family of receptors is probably an important target for mechanically derived signals. $P2X_7$ receptor knockout mice have an osteopenic phenotype that resembles the skeleton of animals subjected to chronic disuse. $P2X_7$ signaling is important for promoting osteoblastic activity and bone formation, whereas $P2X_7$ signaling suppresses osteoclastic bone resorption [41].

The ultimate effect of released prostaglandins on bone biology involves a tangled web of interactions that is extremely complicated. Prostaglandins might (1) recruit new osteoblasts from marrow stroma, (2) amplify their own release by stimulating expression of prostaglandin synthases, (3) improve cell-to-cell communication through cellular gap junctions, (4) reduce apoptosis in osteoblasts, and (5) amplify the loading-related increase of osteoblastic expression of matrix proteins. It is exceedingly difficult to sort out the relative importance of each of these effects.

The two most active prostaglandins (PG) in bone cells are PGE_2 and PGI_2. Both are released from osteoblasts or osteocytes shortly after mechanical loading and have numerous effects on bone, including the recruitment of osteoblasts from marrow stroma [42]. Exogenous PGE_2 administered in rats is strongly osteogenic and results in increased recruitment of osteoblasts and accelerated osteoblastic activity [43]. The E family of prostaglandins also have the ability to amplify their own production [35,44]. This autoamplification effect is mediated through the EP_1 prostaglandin receptor [44], indicating that EP_1 is linked to expression of prostaglandin synthase. However, the anabolic effects of PGE_2 are mediated through the EP_4 prostaglandin receptor [45], suggesting that signaling downstream from EP_4 is important in bone matrix synthesis. Prostaglandin signaling through the EP_2 receptor improves cell-to-cell communication through cellular gap junctions [46–48], and prostaglandins reduce apoptosis in osteoblasts [49] by inhibiting caspase 3.

There are two isoforms of prostaglandin synthase (cyclooxygenase), constitutive (COX-1) and inducible (COX-2). Selective inhibition of COX-2 using NS-398 is considerably more effective in blocking loading-induced bone formation in vivo than is indomethacin, which blocks both isoforms of cyclooxygenase [50,51]. Loading of bone cells causes rapid prostaglandin release from cells and increased expression of COX-2 about 1 h after loading [32]. Nonsteroidal antiinflammatory drugs (NSAIDs) given before mechanical loading suppress loading-induced expression of early-response genes such as c-*fos* [52]. Administration of NS-398 3 h before mechanical loading suppressed bone formation by 67% in the rat tibia, whereas administration of the drug 30 min after loading had no significant effect [52]. These findings demonstrate that prostaglandin synthesis is most important before loading, suggesting that prostaglandins must be available at the time of loading to potentiate the osteogenic response.

Nitric oxide release from bone cells appears to be involved in cellular mechanotransduction because the nitric oxide synthesis inhibitor L-NAME (N^{ω}-nitro-L-arginine methylester) suppresses mechanically induced bone formation in vivo [53]. The endothelial isoform of nitric oxide synthase (NOS-3) is thought to mediate the effects of mechanical forces in bone tissue [37]. However, it is not clear how nitric oxide affects intracellular signaling pathways in osteoblastic cells. In other cell types, nitric oxide binds to soluble guanylyl cyclase, thus stimulating the enzyme and increasing intracellular cyclic guanosine monophosphate (GMP). Cyclic GMP has a number of effects in different cell types and has been suggested as a mediator of mechanical loading in osteoblastic cells [54]. Alternatively, nitric oxide may have a more important role as a mediator of the suppressive effects of mechanical loading on osteoclasts. Nitric oxide is known to be a strong inhibitor of osteoclast activity [55–57] and has been shown to decrease expression of receptor activator of NFkB ligand (RANKL, a.k.a., osteoclast differentiation factor) and increases expression of osteoprotegerin (OPG, an inhibitor of osteoclast differentiation), which in turn leads to decreased recruitment of osteoclasts [58]. Therefore, it appears that local release of nitric oxide enhances bone formation and suppresses bone resorption, suggesting that nitric oxide potentiates an anabolic response.

Conclusions

Exercise can build bone strength by activating the intrinsic mechanotransduction machinery that directs bone formation to where it is most needed. We are beginning to understand the important role desensitization plays in mechanotransduction. Experimental protocols that insert "rest" periods to reduce the effects of desensitization can double anabolic responses to mechanical loading. Several key pathways are emerging from skeletal mechanotransduction research including membrane ion channels, ATP signaling, and second messengers such as prostaglandins and nitric oxide.

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