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COMMENTARY

Synergies at the synapse

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Genetically engineered mice continue to provide insights into the details of physiology beyond what investigators might have originally expected. Despite the formal possibility that gene 'knockout' or other targeted gene manipulations might lead to perturbed development and highly confounded interpretations of changes in physiology measured in adult mice, these limitations are not routinely faced following perturbation of genes encoding G protein-coupled receptor (GPCR) systems. In fact, it has been reasonably straightforward to interpret the findings in animals where mutation or deletion of genes encoding GPCRs have been undertaken. Perhaps this is because GPCRs typically effect modulatory roles–not regulating on – off switches (except perhaps in sensory systems), but changing the signal frequency or intensity of ongoing cellular activity.

One example where gene targeting has had a profound impact is on the understanding of the sympathetic nervous system, in general, and for the roles of α_2-adrenoceptor (AR) subtypes, in particular. There are three subtypes of α_2-ARs (dubbed α_{2A}-, α_{2B}-, and α_{2C}-AR). These receptor subtypes are encoded by three distinct, intronless genes that exist on each of three chromosomes in human beings as well as in mice.

Mutation of the α_{2A}-AR to D79N α_{2A}-AR or creation of null alleles for the α_{2A}-, α_{2B}-, and α_{2C}-AR subtypes has revealed the roles of these subtypes in various physiological and behavioral paradigms (Link *et al*., 1996; MacMillan *et al*., 1996; Lakhlani *et al*., 1997; Rohrer & Kobilka, 1998; Altman *et al*., 1999). The α_{2A}-AR appears to be the subtype that plays the principal role in response to α_2-agonists for the suppression of blood pressure, attenuation of pain perception, analgesia, anesthetic sparing, and suppression of neurotransmitter release (MacMillan *et al*., 1996; Lakhlani *et al*., 1997; Altman *et al*., 1999). In contrast, it is the α_{2B}-AR subtype that elicits the pressor effect of α_2-agonists and, as well, plays a role in salt-induced hypertension (Link *et al*., 1996; Rohrer & Kobilka, 1998). The α_{2C}-AR subtype, as will be discussed below, also contributes to the suppression of neurotransmitter release and, in addition, has effects on behavioral systems, such as altering the swimming behavior of mice – an experimental paradigm for testing the efficacy of antidepressant agents (Rohrer & Kobilka, 1998).

As in any other area of scientific exploration, the more rigorous the biological assessment, the greater the detail and understanding achieved by the studies. An elegant example of this is in the studies of Hein *et al*. (1999), who demonstrate in $\alpha_{2A/C}$-AR 'double knockout' mice that both the α_{2A}- and α_{2C}-AR subtypes contribute to the regulation of neurotransmitter release and do so in a frequency-dependent fashion. Thus, it is the α_{2A}-AR that suppresses norepinephrine in response to high-frequency and high-intensity electrical stimulation, whereas the α_{2C}-AR attenuates norepinephrine release at lower stimuli and frequency intervals. In fact, the role of the α_{2C}-AR is appreciated only when both the α_{2A}- and α_{2C}-AR subtypes have been eliminated (Hein *et al*., 1999). The reason that the loss of both receptor subtypes must occur for the α_{2C}-AR role in the suppression of neurotransmitter release to be detected is not clear. One wonders if hetero-oligomers of α_2-AR subtypes might occur in cells where both α_{2A}- and α_{2C}-AR subtypes are expressed, and have unexpected influence in α_{2C}-AR function, by analogy with hetero-oligomers of opioid receptor subtypes (Jordan & Devi, 1999).

The paper by Trendelenburg *et al*. (2003) in this issue of the *British Journal of Pharmacology* represents a continuation of rigorous analysis of the interplay of G_i/G_o-coupled receptors (such as the α_2-AR autoreceptor) and G_q-coupled receptors on tritium overflow from tissues pre-equilibrated with ^{3}H-noradrenaline. The present findings follow up on earlier observations that angiotensin and bradykinin enhance tritium overflow from mouse atria by extending these studies to vas deferentia stimulated under various degrees of α_2-AR inhibition (Cox *et al*., 2000). These studies were devised to explore in more detail the crosstalk between angiotensin (AT_1) and bradykinin (B_2) receptors, where it is shown that the G_i/G_o system must be engaged by the α_2-AR in order for the G_q-enhanced stimulation of tritium overflow to be detected. Thus, incubation with antagonists at the α_2-AR (such as phentolamine and rauwolscine) or elimination of α_2-AR autoreceptor function by gene deletion, attenuates and eliminates, respectively, enhancement of tritium overflow by AT_1 and B_2 receptors. This interplay between G_i/G_o- and G_q-coupled receptors is not limited to α_2-ARs, however. In the presence of phentolamine or the absence of α_2-AR gene expression, the effects of AT_1 or B_2 receptor agonists to enhance tritium overflow can be mimicked by engaging G_i/G_o-coupled neuropeptide Y, cannabanoid, or μ-opioid receptors.

The interesting and obvious question arises as to what might represent the molecular bases for the interesting physiological findings observed. The findings are reminiscent of studies summarized in 1993 by Bourne & Nicoll (1993), concerning coincident signaling in neurotransmitter systems. Bourne & Nicoll (1993) postulated that convergence on differing

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isoforms of adenylyl cyclase could explain synergism of G_s- and G_i-coupled pathways, or, alternatively, Ca^{2+}- and G_s-coupled pathways, which are functionally antagonistic of one another when activated individually or in nontemporally linked experimental paradigms, but are synergistic upon paired or coincident activation. These authors proposed that the varying isoforms of cyclase, with different sensitivities to $\beta\gamma$ subunits, Ca^{2+} or coincident regulation by G_s and $\beta\gamma$ subunits, represented the molecular machines that integrated these disparate neuronal inputs. It is unlikely that the enzyme adenylyl cyclase represents the molecular integrating machine in the physiological settings under study, particularly since Starke and colleagues have previously shown that G_s-mediated β-adrenoceptor enhancement of tritium overflow is *in*dependent of engagement of G_i/G_o-coupled receptor systems. Nonetheless, it is possible that a similar molecular integration occurs at the level of the receptor-operated K^+ channels, voltage-gated Ca^{2+} channels, or synaptic vesicle fusion complexes intimately involved in frequency-modulated neurotransmission in the sympathetic nervous system.

The interplay enhancement of tritium overflow by AT_1 and B_2 receptors was observed to occur with engagement of any number of G_i/G_o-coupled receptors tested in these experiments (NPY, μ-opioid receptor, cannabanoid receptor, and α_2-AR). Interestingly, this crosstalk required the α_{2C}-AR, not the α_{2A}-AR, subtype. The observation that it is the α_{2C}-AR subtype that plays the predominant role in determining the extent of tritium overflow in response to G_q-coupled agonists suggests that G_i-coupled changes in membrane potential, particularly via response to low-frequency stimulation (a property of the α_{2C}-AR subtype, in particular; Hein *et al*., 1999), might account for this response.

The findings of Trendelenburg *et al*. implicate a crosstalk between G_q- and G_i/G_o-coupled pathways, which encourages further exploration to reveal its molecular basis – be it receptor oligomers, microcompartments, scaffolds, or integrative 'molecular machines' composed of one or several molecular players. The findings do re-emphasize, however, the intricate molecular awareness of the synaptic junction of just which neurotransmitter receptors, and their effector pathways, are engaged, exquisitely synthesizing biochemical and electrical inputs to achieve appropriate trans-synaptic communication.

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References

ALTMAN, J.D., TRENDELENBURG, A.U., MACMILLAN, L., BERNSTEIN, D., LIMBIRD, L., STARKE, K., KOBILKA, B.K. & HEIN, L. (1999). Abnormal regulation of the sympathetic nervous system in alpha2A-adrenergic receptor knockout mice. *Mol. Pharmacol.,* **56,** 154 – 161.

BOURNE, H.R. & NICOLL, R. (1993). Molecular machines integrate coincident synaptic signals. *Cell,* **72**(Suppl)**,** 65 – 75.

COX, S.L., SCHELB, V., TRENDELENBURG, A.U. & STARKE, K. (2000). Enhancement of noradrenaline release by angiotensin II and bradykinin in mouse atria: evidence for cross-talk between G(q/11) protein- and G(i/o) protein-coupled receptors. *Br. J. Pharmacol.,* **129,** 1095 – 1102.

HEIN, L., ALTMAN, J.D. & KOBILKA, B.K. (1999). Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. *Nature,* **402,** 181 – 184.

JORDAN, B.A. & DEVI, L.A. (1999). G-protein-coupled receptor heterodimerization modulates receptor function. *Nature,* **399,** 697 – 700.

LAKHLANI, P.P., MACMILLAN, L.B., GUO, T.Z., MCCOOL, B.A., LOVINGER, D.M., MAZE, M. & LIMBIRD, L.E. (1997). Substitution of a mutant alpha2a-adrenergic receptor via 'hit and run' gene targeting reveals the role of this subtype in sedative, analgesic, and anesthetic-sparing responses *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 9950 – 9955.

LINK, R.E., DESAI, K., HEIN, L., STEVENS, M.E., CHRUSCINSKI, A., BERNSTEIN, D., BARSH, G.S. & KOBILKA, B.K. (1996). Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. *Science,* **273,** 803 – 805.

MACMILLAN, L.B., HEIN, L., SMITH, M.S., PIASCIK, M.T. & LIMBIRD, L.E. (1996). Central hypotensive effects of the alpha2a-adrenergic receptor subtype. *Science,* **273,** 801 – 803.

ROHRER, D.K. & KOBILKA, B.K. (1998). Insights from *in vivo* modification of adrenergic receptor gene expression. *Annu. Rev. Pharmacol. Toxicol.,* **38,** 351 – 373.

TRENDELENBURG, A.U., MEYER, A., KLEBROFF, W., GUIMARAES, S. & STARKE, K. (2003). Cross-talk between presynaptic angiotensin receptors, bradykinin receptors and alpha(2)-autoreceptors in sympathetic neurons: a study in alpha(2)-adrenoceptor deficient mice. Crosstalk between presynaptic receptors. *Br. J. Pharmacol.,* **138,** 1389 – 1402.

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