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COMMENTARY

Use of knockout technology to resolve pharmacological problems

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Knock-out (KO) mouse technology has given pharmacologists a powerful tool to study function in the absence of selective antagonists or inhibitors. Such KO technology can confirm predicted function, serendipitously reveal unrecognized function, or help define the mode of action of a drug. In this issue, Liles *et al.* demonstrate, employing mice unable to synthesize noradrenaline due to the KO of the dopamine-β-hydroxylase gene, that the sympathomimetic actions of ephedrine are directly, rather than indirectly, mediated. This may end 50 years of debate about the actions of ephedrine.
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Abbreviations: DBH, dopamine-β-hydroxylase; KO, knockout; WT, wild-type

Knock-out (KO) mouse technology has given pharmacologists a way out of the impasse caused by lack of antagonists or inhibitors of sufficient selectivity. KO studies can confirm what we already suspect. For instance, in the area of adrenergic pharmacology, studies of wild-type (WT) and KO mice have confirmed that the α_{2A}-adrenoceptor is involved in the hypotensive actions of α_2-adrenoceptor agonists (MacMillan *et al.*, 1996), and that the α_{1D}-adrenoceptor subtype is involved in pressor responses (Tanoue *et al.*, 2002). KO studies can also throw up serendipitous findings. For instance, postjunctional α_{2A}-adrenoceptors have been shown to mediate contractions in mouse vas deferens (Cleary *et al.*, 2003). KO technology can confirm tentative conclusions which would have been almost impossible to verify with pharmacological agents alone: it was suggested that α_{2A}-adrenoceptors were not the only prejunctional α_2-adrenoceptors (Ho *et al.*, 1998), but only KO technology could confirm the additional presence of α_{2C}- (Altman *et al.*, 1999), and even α_{2B}-adrenoceptors (Trendelenburg *et al.*, 2003). Such studies confirm predicted function or serendipitously reveal unrecognized function. KO technology can also be used to set up a model system to define the mode of action of a drug.

In this issue of the *British Journal of Pharmacology*, Liles *et al.* (2007a) have used KO technology to answer a long-standing question: are responses to l-ephedrine predominantly directly mediated or due indirectly to the release of noradrenaline, or a mixture of both actions. This may not be the first paper to use KO technology in such a way, but is a clear example of the genre. Ephedrine has long been thought of as an indirect sympathomimetic, although debate about its indirect (Fleckenstein and Burn, 1953) or direct (Krogsgaard, 1956) modes of action goes back over 50 years. Ephedrine is a potent substrate for the noradrenaline transporter (Rothman *et al.*, 2003), so that there are cogent reasons for predicting indirect actions.

This study looks at cardiovascular responses in anaesthetized mice (Liles *et al.*, 2007a). Previous studies in anaesthetized rats and isolated tissues have suggested either that the actions of ephedrine were a mixture of direct and indirect actions (Kawasuji *et al.*, 1996; Kobayashi *et al.*, 2003), or that the actions were mainly direct (Liles *et al.*, 2006b). The authors employed mice with KO of the dopamine-β-hydroxylase gene (DBH-KO), unable to synthesize noradrenaline, to answer this question definitively. The pressor and tachycardic responses to tyramine were virtually abolished in anaesthetized DBH-KO mice, demonstrating that tyramine acts virtually exclusively by an indirect mechanism. However, the α-adrenoceptor-mediated pressor response to ephedrine, like those to noradrenaline and phenylephrine, were unaffected by DBH-KO. Likewise, the tachycardia to ephedrine was unaffected by deletion of DBH. Hence, the conclusion is that the actions of ephedrine are directly mediated.

The changes seen in the KO seem so clearly linked to the function of the knocked-out gene that differences in genetic background between KO and WT can presumably be ruled out. Therefore, could adaptive changes due to the KO have confounded the results? This also seems unlikely: there was no evidence of any change in responses to noradrenaline or

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other directly acting agonists. Indeed, the supersensitivity seen to noradrenaline following chemical sympathectomy was not seen in DBH-KO mice, perhaps because of raised dopamine levels (Liles *et al*., 2007a).

Of course, the definitive conclusion from this study, that ephedrine (and more correctly l-ephedrine; what of pseudoephedrine?) is predominantly a directly acting sympathomimetic, applies only to the mouse. It is possible that the situation is different in other species. However, the results of KO experiments give us the confidence to re-examine other experimental systems in a new light.

References

Altman JD, Trendelenburg AU, MacMillan L, Bernstein D, Limbird L, Starke K *et al*. (1999). Abnormal regulation of the sympathetic nervous system in alpha2A-adrenergic receptor knockout mice. *Mol Pharmacol* **56**: 154–161.

Cleary L, Vandeputte C, Docherty JR (2003). Investigation of postjunctional alpha1- and alpha2-adrenoceptor subtypes in vas deferens from wild-type and alpha2A/D-adrenoceptor knockout mice. *Br J Pharmacol* **138**: 1069–1076.

Fleckenstein A, Burn JH (1953). The effect of denervation on the action of sympathomimetic amines on the nictitating membrane. *Br J Pharmacol Chemother* **8**: 69–78.

Ho SL, Honner V, Docherty JR (1998). Investigation of the subtypes of alpha2-adrenoceptor mediating prejunctional inhibition in rat atrium and cerebral cortex. *Naunyn Schmiedebergs Arch Pharmacol* **357**: 634–639.

Kawasuji T, Koike K, Saito H (1996). Chronotropic effects of optical isomers of ephedrine and methylephedrine ion the isolated rat right atria and *in vitro* assessment of direct and indirect actions on beta1-adrenoceptors. *Biol Pharm Bull* **19**: 1423–1428.

Kobayashi S, Endou M, Sakuraya F, Matsuda N, Zhang XH, Azuma M *et al*. (2003). The sympathomimetic actions of l-ephedrine and d-pseudoephedrine: direct receptor activation or norepinephrine release? *Anesth Analg* **97**: 1239–1245.

Krogsgaard AR (1956). The effect of intravenously injected reserpine on blood pressure, renal function and sodium excretion. *Acta Med Scand* **154**: 41–51.

Liles JT, Baber SR, Deng W, Porter JR, Corll C, Murthy SN *et al*. (2007a). Pressor responses to ephedrine are not impaired in dopamine-b-hydroxylase knockout mice. *Br J Pharmacol* **150**: 29–36 (this issue).

Liles JT, Dabisch PA, Hude KE, Pradhan L, Vamer KJ, Porter JR *et al*. (2006b). Pressor responses to ephedrine are mediated by a direct mechanism in the rat. *J Pharmacol Exp Ther* **316**: 95–105.

MacMillan LB, Hein L, Smith MS, Piascik MT, Limbird LE (1996). Central hypotensive effects of the alpha2a-adrenergic receptor subtype. *Science* **273**: 801–803.

Rothman RB, Vu N, Partilla JS, Roth BL, Hufeisen SJ, Compton-Toth BA *et al*. (2003). *In vitro* characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals actions as noreponephrine transporter substrates. *J Pharmacol Exp Ther* **307**: 138–145.

Tanoue A, Nasa Y, Koshimizu T, Shinoura H, Oshikawa S, Kawai T *et al*. (2002). The alpha(1D)-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. *J Clin Invest* **109**: 765–775.

Trendelenburg AU, Philipp M, Meyer A, Klebroff W, Hein L, Starke K (2003). All three alpha2-adrenoceptor types serve as autoreceptors in postganglionic sympathetic neurons. *Naunyn Schmiedebergs Arch Pharmacol* **368**: 504–512.