sites was found. Figure 3 of ref. 1 shows the binding-inhibition curve of unlabelled nitrendipine, determined at the K_D concentration of ^{3}H-nitrendipine. The original data on specific binding (not defined by the authors) are presented in transformed form without giving either the total binding in d.p.m. (or c.p.m. or pmol) or the blank (nonspecific, unsaturable) binding. Accepting the data presented, we have calculated the 50% inhibitory concentration (IC_{50}) and the slope factor of this curve by computer analysis: the former is ~670 nM and the latter ~0.52. In Fig. 3 legend[1] the IC_{50} is reported to be 1 nM, a difference of nearly three orders of magnitude. We considered the possibility of printing errors:

(1) In Fig. 3 of ref. 1 the scale runs from 10^{-9} to 10^{-4} M. If the scale was intended to extend from 10^{-12} to 10^{-7} M, the IC_{50} value in the legend would be correct. However, the slope factor of 0.5 would remain unchanged, which is inconsistent with a single site having a K_D of 1.18 nM.

(2) If the legend should read: "IC_{50} values for nitrendipine and BAY-K-8644 were, respectively, 1 and 50 μM (instead of 1 and 50 nM) the figure legend would be consistent with the figure but not with the text, where nitrendipine bound with a K_D of 1.18 nM.

Alternatively, experimental errors could have been made, which could include the use of partially degraded, unlabelled 1,4-dihydropyridines (which are sensitive to light and oxidation) or dilution errors (see ref. 2). If the binding-inhibition curve for BAY-K-8644 is correct, then according to the printed scale, BAY-K-8644 has an IC_{50} of ~30,000 nM; this is the range in which the authors speculated (see Fig. 1 and text of ref. 1) that BAY-K-8644 acts as a Ca^{2+}-channel blocking agent. These potencies (assuming that the scales in the figures are correct) contradict those reported by Albus *et al.*[3] in PC12 cells: in this study the 50% effective concentration (EC_{50}) of BAY-K-8644 in potentiating ^{3}H-noradrenaline release was 10 nM, close to the K_D (16.3 nM) of BAY-K-8644, determined by inhibition of saturable ^{3}H-nitrendipine binding in membrane fragments from these cells.

Note added in proof: Since the first description of calcium channel-linked 1,4-dihydropyridine receptor sites[4], we have repeatedly emphasized[5] the necessity of demonstrating the appropriate stereoselectivity of these receptors, regardless of the calcium channel subtype[6]. Other sites of lower affinity[4], similar to those found by García *et al.*[1] in their displacement study, have the opposite stereoselectivity and have now been shown to be associated with the nucleoside transporter[7].

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1. García, A. B. *et al. Nature* **309**, 69–71 (1984).
2. Gould, R. J., Murphy, K. M. M. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3656–3660 (1982).
3. Albus, U., Habermann, E., Ferry, D. R. & Glossmann, H. *J. Neurochem.* **42**, 1186–1189 (1984).
4. Bellemann, P., Ferry, D., Lübbecke, F. & Glossman, H. *Arzneimittel-Forsch. Drug Res.* **31** (II), 2064–2068 (1981).
5. Glossmann, H. & Ferry, D. R. *Meth. Enzym.* **109**, 513–551 (1985).
6. Glossmann, H., Ferry, D. R., Goll, A. & Romsbusch, M. *J. cardiovasc. Pharmac.* **6**, S608–S621 (1984).
7. Striessnig, J., Zernig, G. & Glossmann, H. *Eur. J. Pharmac.* (in the press).

GARCÍA *ET AL.* REPLY—We appreciate the interest of Dr H. Glossmann in our letter[1] and thank him for his comments. Our paper was intended to show that the novel dihydropyridine BAY-K-8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) activates voltage-sensitive calcium channels of cat adrenomedullary chromaffin cells; numerous unpublished data and two more recent papers from our laboratory[2,3] suggest strongly that this drug acts on such channels to cause their activation and/or increase their opening time, thereby enhancing Ca^{2+} entry with subsequent potentiation of catecholamine secretory responses, triggered by chromaffin-cell depolarizing stimuli.

The rationale in reaching this conclusion was based on three sets of experimental data: (1) BAY-K-8644 enhanced catecholamine release in response to weak, but not to intense, K^+ depolarizations; (2) the drug increased uptake of ^{45}Ca into isolated chromaffin cells at weak, but not at stronger, K^+ depolarizations; and (3) BAY-K-8644 displaced ^{3}H-nitrendipine bound to adrenomedullary membrane fragments.

Certainly, there is a printing error in Fig. 3 legend of our paper[1]: the IC_{50} values for nitrendipine and BAY-K-8644 were 1 and 50 μM, respectively. These values are much higher than both the EC_{50} of nitrendipine (20 nM) required to inhibit catecholamine release from cultured bovine adrenal medulla cells[4] and the EC_{50} of BAY-K-8644 required to potentiate K^+-evoked catecholamine release from chromaffin and PC12 cells (10–50 nM)[1,5], to increase the peak inward Ca^{2+}-channel current in single guinea pig ventricular cells (50–100 nM)[6] or to increase the K^+-evoked contractions of rat vas deferens (1–10 nM; our unpublished results). Several factors can account for this discrepancy: (1) nitrendipine and BAY-K-8644 bind to different sites on the dihydropyridine receptor, as suggested by the non-competitive antagonism by both drugs of K^+-evoked catecholamine release (Fig. 1*b* of ref. 1) and by the fact that both BAY-K-8644 and nitrendipine bind to high- and low-affinity sites on heart membranes[7,8]. (2) The source of membranes used in our experiments was a crude microsomal fraction, containing large amounts of chromaffin granule membranes. In fact, a rough calculation of the number of ^{3}H-nitrendipine binding sites (Ca^{2+} channels?) per μm^2 of surface area gave less than 1 channel per μm^2, about 0.1 of the number found with patch-clamp studies on bovine adrenal chromaffin cells[9]. (3) Frozen or isolated membranes can contain modified Ca^{2+} channels or channel components having different properties; also, some endogenous substance that modulates the activation of chromaffin-cell Ca^{2+} channels may be lost during membrane isolation[9]. (4) Glossmann suggests that the photosensitive dihydropyridines used in our experiment may be inactivated; this is unlikely because we performed the experiment under a sodium lamp and both nitrendipine and BAY-K-8644 were pharmacologically active when tested on adrenal gland heart and vas deferens. (5) Tissue and species-specific variations in interactions of dihydropyridines with ^{3}H-nitrendipine binding sites have been described[10].

Because novel dihydropyridine calcium channel activators are of great interest for neurosecretory research, we decided to publish our preliminary findings and included radioligand-binding displacement studies as they corroborated our conclusion that BAY-K-8644 was potentiating catecholamine release by acting on calcium channels; it seemed to us that the inconsistency between these data and the effects of the drug on secretion did not justify a delay in reporting our data.

We are presently performing radioligand-binding studies with several dihydropyridines using highly purified chromaffin cell plasma membrane preparations to define the molecular characteristics of such binding sites, their relation to voltage-sensitive Ca^{2+} channels and the number of such sites per cell.

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1. García, A. G. *et al. Nature* **309**, 69–71 (1984).
2. Montiel, C., Artalejo, A. R. & García, A. G. *Biochem. biophys. Res. Commun.* **120**, 851–857 (1984).
3. García, A. G., Sala, F., Ladona, M. G., Ceña, V. & Montiel, C. in *Regulation of Transmitter Function: Basic and Clinical Aspects* (ed. Vizi, E.S. & Magyar, K.) 51–63 (Elsevier, Amsterdam, 1984).
4. Ceña, V., Nicolas, G. P., Sánchez-García, P., Kirpekar, S. M. & García, A. G. *Neuroscience* **10**, 1455–1462 (1983).
5. Albus, V., Habermann, E., Ferry, D. R. & Glossmann, H. *J. Neurochem.* **42**, 1186–1189 (1984).
6. Hess, P., Lansman, J. B. & Tsien, R. W. *Biophys. J.* **45**, 394a (1984).
7. Bellemann, P., Ferry, D. R., Lübbecke, F. & Glossmann, H. *Arzneimittel-Forsch. Drug Res.* **31**(II), 2064–2067 (1981).
8. Janis, R. A., Rampe, D., Sarmiento, J. G. & Triggle, D. J. *Biochem. biophys. Res. Commun.* **121**, 317–323 (1984).
9. Fenwick, E. M., Marty, A. & Neher, E. *J. Physiol., Lond.* **331**, 599–635 (1982).
10. Gould, R. J., Murphy, M. M. & Snyder, S. H. *Molec. Pharmac.* **25**, 235–241 (1984).