Polydipsia after intracranial injections—a property of NGF or a contaminant?

INTRACRANIAL injections of 2.5S NGF in adult rats have been shown to induce intense polydipsia[1]. Subsequent studies from other authors confirmed this finding and, in addition, gave evidence of increased appetite for sodium solutions[2]. The authors of this last article call attention on the similarity between these effects and those elicited by intracerebral renin injections. Previous work from this laboratory in collaboration with the Department of Pharmacology, University of Heidelberg[3], showed that isorenin activity is present in 2.5S NGF preparations and is separated only after laborious additional purification steps. Thus, NGF prepared with the same method as that used for studies on induced polydipsia and sodium appetite still showed considerable amounts of renin-like activity. According to Cozzari *et al.*[3] it was necessary to perform three further carboxymethyl-cellulose chromatographies after the usual procedure to prepare 2.5S NGF, in order to eliminate all renin-like activity previously detectable.

In view of the many effects which have been recently claimed to be caused by NGF intracranial injections[4,5], it seems essential that whenever high doses of NGF are needed to produce a given effect, it should be clearly established that the preparations are entirely free of contaminants. This precaution is of particular importance since NGF has been found to be tightly bound in mouse salivary extracts with other proteins endowed with enzymatic or other biological activities.

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LEWIS *ET AL.* REPLY—Levi-Montalcini is right to draw attention to the possibility that the effects of NGF on the central nervous system may be mediated by portions of the NGF complex other than the β subunit. As we pointed out in our report[1], the marked similarity between the effects of intracranial injections of 2.5S NGF and renin on thirst and sodium appetite suggested that the effects of 2.5S NGF might be mediated by the cerebral isorenin–angiotensin system. Activation of this system is an exceedingly potent stimulus to thirst[2] and sodium appetite[3]. We have since established that 7S NGF[4] has similar effects on thirst and sodium appetite, and that both 2.5S[5] and 7S NGF produced renin-like pressor responses when injected intravenously in intact and nephrectomised rats[6]. The doses of these NGF preparations needed to produce pressor responses are comparable to those used in the standard *in vitro* NGF bioassay[7] and considerably less than the doses of NGF reported to induce growth in the sympathetic ganglia of newborn mice[8]. The pressor responses to 7S and 2.5S NGF were abolished by the angiotensin-converting enzyme inhibitor SQ 14,225, the angiotensin-receptor blocker saralasin, and NGF antiserum[6]. The same procedure also blocked the effects of these NGF preparations on thirst and sodium appetite[9]. Immunogenically pure β-NGF subunit[10] from the mouse submandibular gland was devoid of pressor activity and had a much smaller and more variable effect on thirst and sodium appetite than other preparations of NGF[6].

Our demonstration that NGF-induced thirst and sodium appetite are mediated through the formation of angiotensin II (AII) raises the possibility that other effects of NGF preparations are similarly mediated. For example, we have recently found that intracranial administration of renin, like 2.5S[11,12] and 7S NGF, results in a marked increase in the activity of ornithine decarboxylase (ODC) in brain and liver[6]. These increases in ODC activity in response to renin and NGF are blocked by pretreatment with SQ 14,225 and are therefore dependent on the formation of AII[6]. The AII-dependent induction of ODC in the liver following NGF administration is due to activation of the pituitary–adrenal axis, as the induction is blocked by either hypophysectomy or adrenalectomy[12]. Otten *et al.*[13] recently reported that systemic injection of 2.5S NGF, the purity of which was controlled by SDS-polyacrylamide gel electrophoresis[14], caused a rapid increase in plasma ACTH and corticosterone levels. However, systemic or central administration of AII also causes an increase in plasma ACTH and corticosteroid levels[15–17]. Therefore, activation of the pituitary–adrenal axis following NGF administration may be due to the presence of a renin-like enzyme in the NGF.

Our findings complement those of Cozzari *et al.*[18], who reported that NGF preparations from mouse submandibular gland are associated with a renin-like enzyme that can only be removed after much additional purification. Whether this renin-like enzyme or other enzymes[19,20] should be regarded as integral components of mouse submandibular gland NGF complexes has not been resolved. However, unless renin-free preparations of NGF are used, the use of angiotensin antagonists such as SQ 14,225 and saralasin should be considered obligatory, particularly when a biological effect attributed to NGF is known to be produced by renin or AII.

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Evolution by gene duplication in insecticide-resistant *Myzus persicae*

THE simple model of "a succession of tandem duplications of the structural gene", proposed by Devonshire and Sawicki[1], cannot account as it stands for the apparent regularity of the variation in enzymatic activity of the organophosphorus (Op) insecticide resistance associated esterase (E4[1] or RAE[2]) observed in specimens of the peach potato aphid, *Myzus persicae*, from different laboratory stock clones, because the esterase does not have the same electrophoretic mobility or phenotype in all the clones compared.

The enzyme from the predominant Op resistant clone in British field populations[3] (E4(MS1G) or RAE(+)[2]) stains after electrophoresis in two close but distinct bands, the rear band being fainter and staining more slowly[2]. Aphids with this electrophoretic phenotype, but with higher enzymatic activity on the gel, have also been collected in the field[2], and these could have arisen by duplication of the E4(MS1G) gene, although other explanations are also possible, for example, that they are homozygous for E4(MS1G), or that they are a mutant in which E4(MS1G) has become more resistant to denaturation in gel assay

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