7-Nitroindazole reduces cerebral blood flow following chronic nitric oxide synthase inhibition

Paul A.T. Kelly*, Isobel M. Ritchie, Douglas E. McBean

Department of Clinical Neurosciences, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK
Faculty of Health Sciences, Dietetics and Nutrition, Queen Margaret University College, Clerwood Terrace, Edinburgh EH12 8TS, UK

Abstract

Blood flow and glucose utilization were measured in rat brain after chronic L-NAME treatment followed by acute 7-nitroindazole. Following chronic L-NAME, blood flow was not significantly different from control. Treatment with acute 7-nitroindazole reduced blood flow to the same extent in both chronic saline and L-NAME groups. Glucose utilization was unaffected. These results suggest that residual NOS activity in brain is sufficient to provide tonic, NO-dependent cerebrovascular dilator tone.

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Acute treatment with N\textsuperscript{G}-substituted arginine analogues inhibits nitric oxide synthase (NOS) and produces widespread vasoconstriction \[3,5\]. This is manifest as both an increase in peripheral arterial blood pressure and a decrease in local cerebral blood flow (lCBF) \[11,12\]. Repeated daily injection with these arginine analogues, inhibits nitric oxide synthase (NOS) activity in the brain by 95% or more \[1,2\], but we have previously shown that over several days of such treatment with nitro-L-arginine methyl ester (L-NAME), ICBF returns towards normal control levels whilst mean arterial blood pressure remains elevated \[8\]. Moreover, despite the almost total inhibition of NOS activity, a further acute challenge with L-NAME causes a reduction in ICBF. One possible explanation for this apparently paradoxical response is that measurements of NOS activity in the brain reflect predominantly neuronal NOS (nNOS) and cerebrovascular endothelial NOS (eNOS) reacts differently to chronic L-NAME treatment. Alternatively, cerebral blood vessels may become more sensitive to the 5% of residual NO which may still be produced. The purpose of this study was to examine the effects of the relatively selective nNOS inhibitor 7-nitroindazole (7-NI) \[13\] upon ICBF and glucose utilization (ICMRglu) in rats previously treated chronically with L-NAME. If the recovery of cerebrovascular dilator tone following chronic L-NAME is an eNOS phenomenon acute 7-NI should have no effect, but if the blood vessels of the brain have become more sensitive to NOS of whatever source, 7-NI should produce a reduction in ICBF.

Rats were injected with L-NAME (75 mg/kg i.p.; \(n=20\)) or saline (\(n=20\)) once daily for 10 days. We have previously shown that this treatment results in a reduction in cerebral NOS activity of at least 95% \[1,9\]. Fifteen hours after the final L-NAME injection, the rats were treated with a single acute dose of either 7-NI (25 mg/kg i.p.; \(n=20\)) or oil (\(n=20\)), and ICBF or ICMRglu were measured from brain tissue samples as described by us previously \[10\]. Data (presented as mean±S.D.) were analysed using Dunnett’s \(t\)-test to allow comparisons for each measurement between the chronic saline/acute oil treated control group and the three drug treatment groups. Acceptable levels of significance were set at \(P<0.05\).

In rats treated chronically with L-NAME, mean arterial blood pressure (MABP; mmHg) increased significantly to
172±10 (cf. 138±7 in controls). Acute treatment with 7-NI had no significant effect on MABP in rats treated chronically with either saline (130±12) or l-NAME (172±18). There were no significant differences in any of the other measured physiological variables (pCO₂, pO₂, pH, rectal temperature, plasma glucose) between control and drug-treated groups.

In keeping with our previously published data, mean lCBF in the chronic l-NAME group was not significantly different from control although small decreases were apparent (between −5% in hippocampus and −12% in frontal cortex) (Table 1). Acute 7-NI, following chronic saline treatment, resulted in significant decreases in lCBF from control ranging from −27% in hippocampus to −37% in frontal cortex. In rats treated chronically with l-NAME, acute 7-NI injection produced significant decreases in lCBF (−28% in hippocampus and −33% in frontal cortex) which were remarkably similar, or even identical to those observed in the chronic saline group.

There were no significant differences in lCMRglu between control and treated groups in any region of the brain analysed in this study. The overall relationship between mean lCBF and mean lCMRglu was very similar mised, irreversible damage to brain tissues is likely to occur [7,18]. The oligoemia which is produced by acute l-NAME, in the absence of any parallel reduction in lCMRglu, is therefore likely to be physiologically unsustainable in the long-term without evoking pathological consequences.

This re-establishment of dilator tone to the cerebral blood vessels appears be unique to the cerebral circulation. Moreover, the adaptive dilatation appears to involve NO, despite the fact that in our hands the chronic l-NAME treatment has been shown to reduce cerebral NOS activity by 95% or more [1,9]. Acute 7-NI following chronic l-NAME treatment, produces a reduction in lCBF to levels which are similar to those found when the drug is given to rats treated chronically with saline. This compares with a somewhat attenuated response to acute l-NAME in chronically treated animals [8], but taken together with that previous study, these results suggest that the residual NOS activity found in brain homogenates (approximately 5%) may be sufficient to provide tonic, NO-dependent cerebrovascular dilator tone in vivo, some of which at least may be of neuronal origin. However, we cannot say that other

| Table 1 | Effects of chronic l-NAME and acute 7-NI treatments upon local cerebral blood flow* |
|-------------------|-------------------|-------------------|-------------------|
| **Control** | **Drug treatments** | **Control** | **Drug treatments** |
| | Chronic saline+acute oil | Chronic saline+acute 7-NI | Chronic l-NAME+acute oil | Chronic l-NAME+acute 7-NI |
| Frontal cortex | 120±9 | 76±8* | 106±14 | 80±12* |
| Parietal cortex | 135±6 | 95±4* | 123±18 | 91±9* |
| Occipital cortex | 94±14 | 66±3* | 85±13 | 67±7* |
| Striatum | 118±13 | 78±7* | 109±11 | 78±8* |
| Hippocampus | 81±6 | 59±5* | 77±14 | 58±6* |

*Data are presented as mean (ml/100 g per min)±standard deviation (S.D.) with n=5 rats in each group. *Significantly different from control group (P<0.05).
adaptive mechanisms may not also be exerting an influence, and nor can we discount the possibility that both \(\text{L-NAME}\) and \(\text{7-NI}\) may be less selective in their effects upon the brain and its circulation than had previously been understood \[6,14,16\].

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References


