Noradrenaline excitation of cortical neurons: A reply

Recently, Stone (1972) has argued that the excitant actions of iontophoretically applied noradrenaline on neurons in the feline cerebral cortex cannot be explained simply on the basis of the pH of the noradrenaline solution in the micropipette, as suggested by Frederickson, Jordan & Phillis (1971). Stone raised several objections to this explanation, the most important being his finding that noradrenaline solutions at pH 3·0, 4·0, and 5·0 had comparable excitant actions on 12 neurons in three urethane anaesthetized rats. Stone (1971) has proposed an alternative explanation for excitant effects of noradrenaline, namely that this agent can cause vasoconstriction of small blood vessels in the vicinity of the micropipette tip with a subsequent excitation of cortical neurons as a result of hypoxia.

It is always difficult to compare results obtained in different species under differing conditions of anaesthesia, and we would like to present some of our own results obtained in rats anaesthetized with methoxyflurane and nitrous oxide and to compare these with results from cats under comparable anaesthetic conditions. We have studied the effects of noradrenaline, applied from a solution at pH 5·0, on a total of 67 neurons in 8 rats. Of these, 44 cells (66%) were depressed by noradrenaline and 23 (34%) were unaffected. No excitant actions were observed. Seventy-six (73%) of the 104 neurons studied in cats anaesthetized with methoxyflurane and nitrous oxide were depressed by noradrenaline applied from a solution at pH 5·0, and only 2 cells were excited. Furthermore, a highly significant difference ($P < 0{\cdot}0025$) was observed between the effects of noradrenaline applied from solutions at pH 4·0 and 5·0 in methoxyflurane-nitrous oxide anaesthetized cats, with 16 out of 107 cells excited when solutions at pH 4·0 were used, and only 2 of 104 cells excited when solutions at pH 5·0 were used. These results, obtained in two species, clearly support our contention that the excitant effects of noradrenaline solutions on cerebral cortical neurons can be virtually eliminated by using solutions at pH 5·0.

It is possible that the disparity between our results in rats and those obtained by Stone are a result of the latter's use of urethane anaesthesia. According to Berndt, Berger & others (1972), ventilation rate and respiratory frequency are distinctly diminished in cats anaesthetized with chloralose-urethane, and compensatory respiratory responses to csf pH changes are also reduced as compared to *cerveau isolé* cats. When these effects are sufficiently pronounced, respiratory acidosis ensues which is reflected in the csf and brain interstitial fluid (cf., Posner, Swanson & Plum, 1965; Huang & Lyons, 1966; Roncoroni, Roehr & Adaro, 1970). As pointed out by Fencl, Miller & Pappenheimer (1966), csf and brain interstitial fluid are poorly buffered, and buffering power is reduced in acidosis. A reduced buffering capacity of brain interstitial fluid could account for the excitations observed by Stone in urethane anaesthetized animals using noradrenaline solutions with low hydrogen ion concentrations (pH 4·0 and 5·0).

Evidence favouring the importance of a role for hydrogen ions in excitations produced in the cortex may also be derived from pH controls. Although Stone (1972) has interpreted the pH controls performed by Krnjević & Phillis (1963) as revealing the response of cortical neurons to hydrogen ions *alone*, the latter authors clearly stated that concentrated NaCl solutions at various pH values were used in their studies. It is evident, therefore, that sodium ions were competing with hydrogen ions for access to the electrode tip in their solutions. We have recently performed experiments in which hydrogen ions alone were ejected from HCl solutions at pH 3·0, 4·0 and 5·0. Excitation of cortical neurons in decerebrate cats was observed at all three pH values. The response was slow of onset, and closely resembled the type of response that Johnson, Roberts & others (1969) interpreted as excitant effects of noradrenaline.

Stone (1971) maintains that the response of small vessels to catecholamines is greatly reduced during methoxyflurane anaesthesia (Baez & Orkin, 1963), thus accounting for the lack of excitation observed in methoxyflurane anaesthetized animals (Phillis & York, 1967) and supporting his contention that the excitant effects observed for noradrenaline in other preparations are due to vasoconstrictor properties of iontophoretically applied agent. This argument is untenable, since we have found significantly more excitations with noradrenaline at pH 4·0 in methoxyflurane-nitrous oxide anaesthetized cats than in *cerveau isolé* animals (Frederickson, Jordan & Phillis, to be published). If the excitations were due to the vasoconstrictor properties of noradrenaline, these should have occurred more frequently in the *cerveau isolé* animals.

The final point made by Stone (1972) is that Boakes, Bradley & others (1971) have shown that (−)-noradrenaline produced excitation of brain stem neurons while the (+)-isomer did not, when both solutions were at the same pH, although he omitted to mention that the (+)-isomer excited 8 of the 21 neurons excited by the (−)-isomer. Boakes & others (1971) used solutions at pH 5·0—6·0, which would be within the acceptable range for cortical neurons, and it must be assumed that these excitations represent genuine actions of noradrenaline. In any case, it is difficult to explain the observation that many neurons were excited by (+)-noradrenaline on the basis of the vasoconstriction hypothesis, since Allen, Rand & Strong (1972) have shown that the (−)-isomer is 669 times more potent than the (+)-isomer as a vasoconstrictor.

The results described here and in other publications (Frederickson & others, 1971; Jordan Frederickson & others, 1972) clearly show that hydrogen ions applied iontophoretically from drug solutions or from solutions of HCl can cause excitation of neurons, and that such hydrogen ion effects are responsible for a major portion of the excitation observed in the cerebral cortex when noradrenaline is applied from "acidic" solutions. The proposal by Stone (1971) that vasoconstriction is responsible for the excitatory actions occurring in response to noradrenaline application to cortical neurons does not appear to be a viable explanation for these effects either in *cerveau isolé* or methoxyflurane-nitrous oxide anaesthetized animals.

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Absence of potentiation of phenylephrine-induced cardiac necroses by theophylline. Selective inhibition by dihydroergocryptine and nicergoline

In rats the cardiac necrotizing activity of low doses of isoprenaline is competitively inhibited by β-adrenoceptor blocking agents (Dorigotti, Gaetani & others, 1969) and is potentiated by theophylline as shown by Martorana (1971) who has suggested a mediatory role for cyclic AMP in the isoprenaline-induced cardiac necroses.

We now report that focal necroses induced by a pure α-adrenoceptor agonist phenylephrine, are not potentiated by theophylline but inhibited selectively by the α-adrenoceptor blocking agents dihydroergocryptine and nicergoline.

The experimental procedure was as described by Dorigotti & others (1969) and by Martorana (1971).

Twenty-four h after administration of phenylephrine the hearts of the treated rats showed focal isoprenaline-like necroses consisting of loss of cross-striation, fragmentation and marked vacuolization of the muscle fibres, infiltration of mononuclear inflammatory cells and of leucocytes.

The percentage of animals with lesions was related to the dose of phenylephrine at least in the range from 1 to 6 mg/kg (s.c.).

The two α-adrenoceptor blocking agents dihydroergocryptine and nicergoline (Arcari, Dorigotti & others, 1968), when administered shortly before the agonist, reduced the incidence of lesions in a dose-dependent way. In the presence of antagonists there was a shift to the right of the dose-response curves and the ED50 of phenylephrine increased many times (Table 1).

In contrast to these findings, high doses of propranolol had no antagonizing action to phenylephrine, while neither dihydroergocryptine nor nicergoline protected the rats from isoprenaline, in accordance with our previous results.

Theophylline, administered as aminophylline, was tested in several groups of rats according to Martorana (1971) but failed to potentiate the responses to 0·5 to 2·5 mg/kg of phenylephrine even at high doses (75 and 150 mg/kg) which themselves induced necroses in the same animals. In control experiments, isoprenaline was fully potentiated as found by Martorana (1971).

Table 1. *Dose of phenylephrine producing cardiac lesions in 50% of the animals (ED50) in the presence of α-adrenoceptor blocking agents administered s.c., 20 min before the agonist.*

Antagonist and dose	ED50 (mg/kg)	Straight line equation
Phenyleprhine alone	2·45 (1·8– 3·48)*	y = 3·31 + 4·25 x
Dihydroergocryptine 1 mg/kg	20·25 (10·82–37·91)*	y = 3·01 + 1·52 x
Dihydroergocryptine 5 mg/kg	31·30 (18·79–52·13)*	y = 1·09 + 2·61 x
Nicergoline 2·5 mg/kg	7·13 (3·15–16·15)*	y = 0·52 + 5·25 x
Nicergoline 25 mg/kg	52·36 (25·85–103·60)*	y = 5·44 + 6·07 x

* Confidence limits per $P = 95\%$.