100 and 120 mm Hg. Grade II hypercapnia produced a 2fold increase in $P_{CO_2}$.

Cortisol output rose more abruptly and fell more slowly during hypercapnia than hypoxia (figure 1). However, the highest values recorded during both grades of hypercapnia were comparable with those during grade I hypoxia. Similar rates of secretion occur in response to infusions of supramaximal doses of ACTH[6] indicating that both stimuli can elicit a maximal secretory response from the adrenal cortex. The pattern of secretion of corticosterone was the same as that of cortisol but the amounts released were less.

Comparatively trivial amounts of catecholamines were released from the adrenal medulla during hypoxia or grade II hypercapnia. In contrast, grade I hypercapnia caused the release of quite substantial amounts of noradrenaline together with smaller but significant amounts of adrenaline (figure 2). This pattern of release differs from that which occurs in response to intense hypoxia in the conscious calf when adrenaline is invariably the predominant amine and may be secreted at a rate of up to 18,000 ng/kg$^{-1}$ min$^{-1}$[7].

These results show that a maximal adrenal cortical response can occur in these animals in response to both hypoxia and hypercapnia of insufficient intensity to elicit significant release of catecholamines from the adrenal medulla. It is concluded that the sensitivity of the pituitary-adrenal cortical axis far exceeds that of the adrenal medulla to both these stimuli in the conscious calf.

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## Effects of ibotenic acid, quisqualic acid and their relatives on the excitability of an identifiable giant neurone of an African giant snail (Achatina fulica Férussac)

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*Summary.* An identifiable giant neurone, PON (periodically oscillating neurone), of Achatina fulica Férussac, inhibited by erythro-β-hydroxy-L-glutamic acid, was also inhibited by 2 relatives of β-hydroxy glutamic acid, ibotenic acid and quisqualic acid. These substances similarly showed the effect on the neurone even in the chloride-free medium.

Takemoto et al.[2–4] isolated a heterocyclic amino acid (ibotenic acid, α-amino-3-oxo-4-isoxazoline-5-acetic acid) which shows a fly-killing effect, from the fungus, Amanita strobiliformis (Paul.) Quer. They[5,6] also isolated another heterocyclic amino acid (tricholomic acid, α-amino-3-oxo-isoxazolidine-5-acetic acid) having the same effect from another fungus, Tricholoma muscarium Kawamura. Quisqualic acid [β-(3, 5-dioxo-1, 2, 4-oxadiazolidin-2-yl)-L-alanine], of which was an anthelminthic effect (anti-Ascaris) demonstrated[7], was isolated also by Takemoto et al. from Quisqualis Fractus[8–10].

In the present study, we attempted to examine effects of these biologically active heterocyclic amino acids and their relatives on the excitability of a giant neurone (the PON, periodically oscillating neurone) identified in the subesophageal ganglia of Achatina fulica Férussac. We demonstrated previously[11,12] that the PON was inhibited remarkably by β-hydroxy glutamic acid (BHGA, especially erythro-L-type), the chemical structure of which resembles those of the heterocyclic amino acids isolated by Takemoto et al., although L-glutamic acid did not affect the same neurone.

Experimental methods were described in detail in the preceding papers[12,13]. In the present study, we examined the effects of the above-mentioned heterocyclic amino acids, not only in the physiological medium but also in the chloride-free condition. To obtain the latter condition, we perfused the dissected ganglia with the chloride-free solution (replaced with acetate) for more than 1 h.

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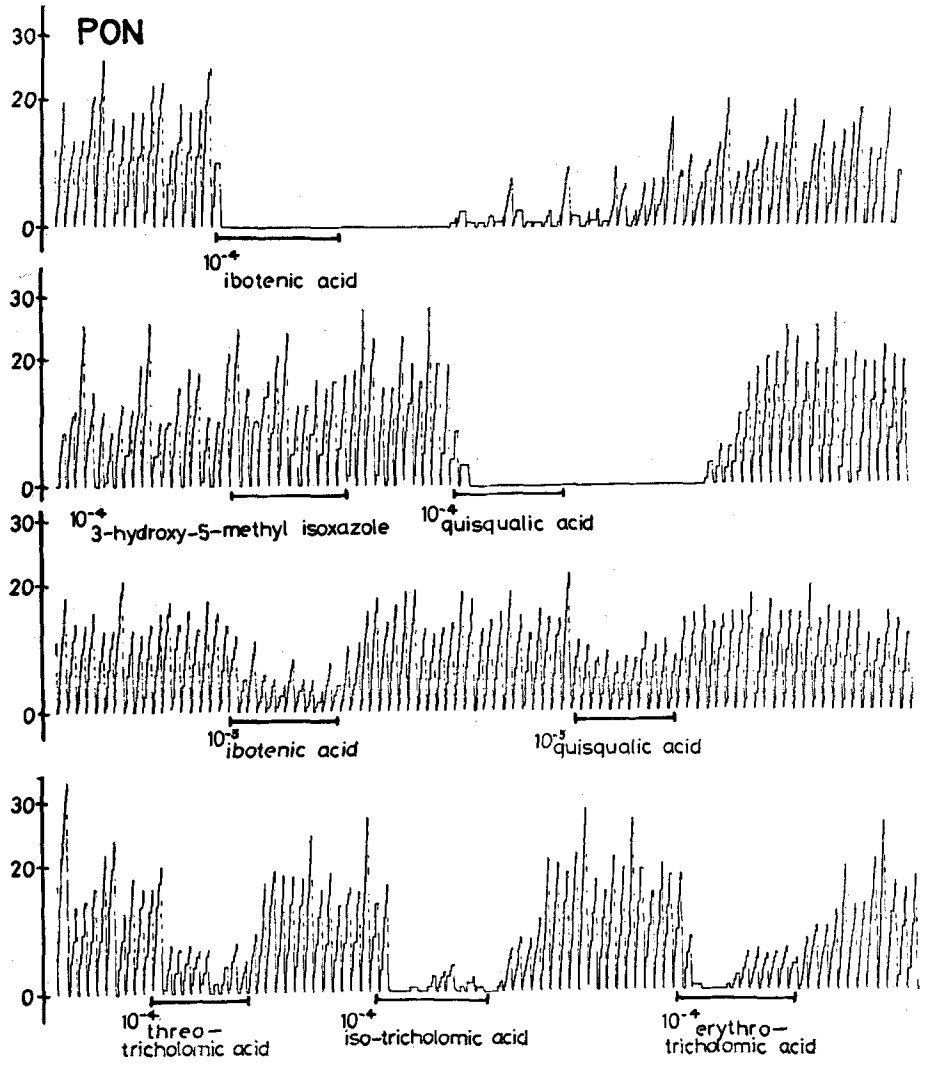


Fig. 1. Inhibitory effect of ibotenic acid, quisqualic acid and 3 isomers of tricholomic acid on PON (periodically oscillating neurone) excitability in the physiological condition (bath application). 4 traces were recorded continuously. Ordinate, the number of spike discharges per min. Abscissa, time course, each histogram is 1 min. We applied $10^{-4}$ kg/l ibotenic acid, $10^{-4}$ 3-hydroxy-5-methylisoxazole, $10^{-4}$ quisqualic acid, $10^{-5}$ ibotenic acid, $10^{-5}$ quisqualic acid, $10^{-4}$ threo-tricholomic acid, $10^{-4}$ iso-tricholomic acid and $10^{-4}$ erythro-tricholomic acid. Note that ibotenic acid and quisqualic acid at $10^{-5}$ kg/l, and 3 isomers of tricholomic acid at $10^{-4}$ kg/l (bath application) showed an inhibitory effect on the PON excitability.

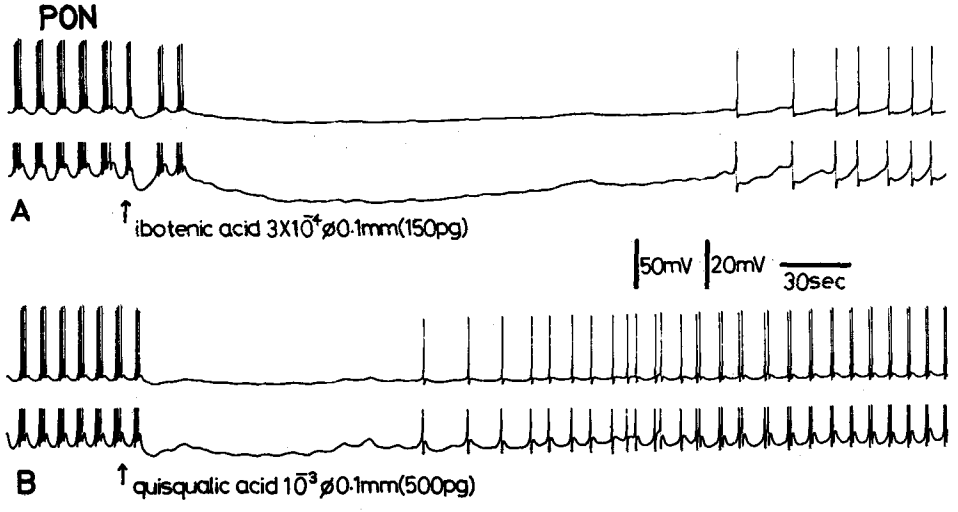


Fig. 2. Effect of ibotenic acid and quisqualic acid on PON excitability in the physiological condition (microdrop application). The upper traces of A and B are the full-spike recordings of the PON biopotential by the pen-writing galvanometer. The lower traces of A and B are the high gain recordings of the upper traces. (The spike peaks have been cut off by an electronic voltage clipper.) In A, a microdrop (100 µm in diameter) of $3 \times 10^{-4}$ kg/l ibotenic acid (the total amount of this substance was about 150 pg) was applied on the PON surface (arrow). In B, a microdrop (the same diameter) of $10^{-3}$ kg/l quisqualic acid (total amount was about 500 pg) was applied on the surface of the same neurone. Note that the microdrop application of ibotenic acid and quisqualic acid caused a remarkable hyperpolarization of the PON neuromembrane, and stopped the spontaneous spike discharges.

Effects of some heterocyclic amino acids on the PON excitability in the physiological condition (bath application)

| No. | Substance | Effect on PON | Examined concentration (kg/l) |
|---|---|---|---|
| 1 | Erythro-$\beta$-hydroxy-L-glutamic acid[11,12] (1) | I | $10^{-6}\sim3\times10^{-6}$* |
| 2 | L-Glutamic acid[11,12] (2) | (–) | $10^{-4}$ |
| 3 | Ibotenic acid (3) | I | $3\times10^{-6}\sim10^{-5}$* |
| 4 | Quisqualic acid (4) | I | $10^{-5}$* |
| 5 | Erythro-tricholomic acid (3) | I | $10^{-4}$* |
| 6 | Threo-tricholomic acid (3) | I | $10^{-4}$* |
| 7 | Iso-tricholomic acid (3) | I | $10^{-4}$* |
| 8 | 3-Hydroxy-5-methyl isoxazole (5) | (–) | $10^{-4}$ |
| 9 | Kainic acid (4) | (–) | $10^{-4}$ |
| 10 | $\alpha$-Allo kainic acid (4) | (–) | $10^{-4}$ |
| 11 | Muscazone (6) | (–) | $10^{-4}$ |
| 12 | 4-Hydroxy pyrrolidone (6) | (–) | $10^{-4}$ |
| 13 | Muscimolnitrile (6) | (–) | $10^{-4}$ |
| 14 | bis-Muscimole (6) | (–) | $10^{-4}$ |
| 15 | 5-Acetamido-3,4-dimethyl-isoxazole | (–) | $10^{-4}$ |
| 16 | 5-Hydroxy-3,4-dimethyl-isoxazole | (–) | $10^{-4}$ |
| 17 | 5-Carboxymethyl-isoxazole | (–) | $10^{-4}$ |
| 18 | 5-Morpholinomethyl-3-isoxazolidone | (–) | $10^{-4}$ |
| 19 | 5-Carboxy-3-methyl-isoxazole | (–) | $10^{-4}$ |
| 20 | 3-Carboxy-5-methyl-isoxazole | (–) | $10^{-4}$ |
| 21 | 2-Oxazolidone | (–) | $10^{-4}$ |
| 22 | 2-Mercapto-5-methyl-2-oxazoline | (–) | $10^{-4}$ |

I, inhibitory effect; (–), no effect; *, critical concentration to produce the effect. (1), donated by Dr M. Kurono of Ono Pharmaceutical Co.; (2), product of Wako Pure Chemical; (3), donated by Dr H. Iwasaki of Takeda Chemical Co.; (4), donated by Prof. Emer. T. Takemoto of Tohoku University; (5), product of Sankyo Co.; (6), donated by Prof. C. H. Eugster of Zurich University.

The results obtained in the physiological medium are summarized in the table. The following heterocyclic amino acids, administered by bath application, showed an inhibitory effect on PON excitability in the physiological condition: ibotenic acid; quisqualic acid; erythro-, threo- and iso-tricholomic acid. As shown in figure 1, the inhibitory effect of ibotenic acid and quisqualic acid is stronger than that of three isomers of tricholomic acid. The critical concentrations of these substances to produce the effect were respectively: ibotenic acid ($3\times10^{-6}$ to $10^{-5}$ kg/l), quisqualic acid ($10^{-5}$ kg/l) and 3 isomers of tricholomic acid ($10^{-4}$ kg/l). The effect disappeared after washing the ganglia with the physiological solution. Substances examined in the present study other than the 5 above-mentioned amino acids had no effect on the PON excitability in a concentration of $10^{-4}$ kg/l.

We tried the local application of the 2 effective substances, ibotenic acid and quisqualic acid, on the PON in the physiological condition (figure 2). A microdrop (about 100 $\mu$m in diameter) of the solution of each substance was placed on the PON surface (the diameter of the neuronal soma is about 200 $\mu$m) under a microscope. Several seconds after the application, the PON biopotential hyperpolarized and stopped the spike discharges. The effect of these substances, applied in this way, was also reversible. We conclude that the inhibition of the PON biopotential caused by the 2 substances is due to the hyperpolarization of the PON neuromembrane.

To determine whether the hyperpolarizing effect of the 2 heterocyclic amino acids on the PON neuromembrane might be due to the increase of the membrane permeability to chloride ions, we tried the microdrop application of the 2 substances on the PON surface in the chloride-free medium. As shown in figure 3, the 2 substances showed the hyperpolarizing effect on the PON

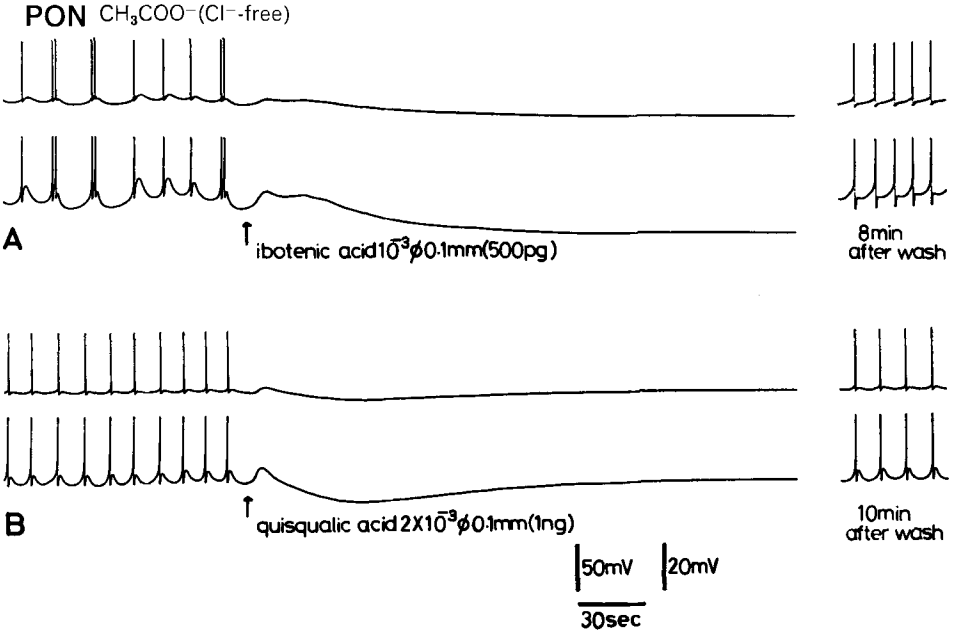


Fig. 3. Effect of ibotenic acid and quisqualic acid on PON excitability in the $Cl^-$-free condition (microdrop application). Chloride ions were replaced with acetate ions in the environmental solution. The upper traces of A and B are the full-spike recordings of the PON biopotential. The lower traces of A and B are the high gain recordings of the upper traces. (The spike peaks have been cut off by an electronic voltage clipper.) In A, a microdrop (100 $\mu$m in diameter) of $10^{-3}$ kg/l ibotenic acid (the total amount of this substances was about 500 pg) was applied on the PON surface (arrow). In B, a microdrop (the same diameter) of $2\times10^{-3}$ quisqualic acid (the total amount was about 1 ng) was applied (arrow). Note that the 2 substances showed the inhibitory effect on the PON excitability even in the chloride-free medium.

neuromembrane in the chloride-free condition, in the same manner as in the physiological medium. We confirmed that β-hydroxy glutamic acid (BHGA, erythro-L-type) also inhibited the PON in the chloride-free condition. We conclude that the inhibitory effect of these substances on the PON is probably not due to the membrane permeability increase to chloride ions.

Walker et al.[14] reported that L-glutamic acid and ibotenic acid show the same effect (inhibitory or excitatory) on identifiable giant neurones of a European garden snail (Helix aspersa). They suggested that the 2 amino acids will act on the same receptor of the neuromembrane under the same ionic mechanism (their inhibitory effect is due to the membrane permeability increase to both chloride and potassium). Concerning the PON of Achatina fulica Férussac, not L-glutamic acid, but erythro-β-hydroxy-L-glutamic acid and 2 heterocyclic amino acids (ibotenic acid and quisqualic acid) show the same effect. We suggest the possibility that the 3 substances act on the same sites of neuromembrane under the same ionic mechanism. Unlike the results of Walker et al. using Helix aspersa, the 3 amino acids are not considered to increase the PON membrane permeability to chloride.

Lea and Usherwood[15,16] suggested that the conductance increase of the locust (Schistocerca gregaria) muscle fibre membrane caused by ibotenic acid is due to the membrane permeability increase of chloride. Their findings on the effect of ibotenic acid would be different from the case of the PON neuromembrane.

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## The effect of vinblastin and vincristin on single nerve fibres

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*Summary.* The perfusion of the node of Ranvier with vinblastin or vincristin reduces the amplitude of the action potential within a few seconds. Vincristins is 10fold more active than vinblastin. Upon withdrawal, the effect is promptly reversible and it is antagonized by acetylcholine.

Vinblastin and vincristin, the 2 main alkaloids of Vinca rosea, are endowed with a marked neurotoxicity even at current therapeutical doses[1]. It has been maintained that the primary alteration is an impairment to the axonal transport through degenerative changes in nervous tissue[2]. The propagation of the action potentials would be altered only secondarily, some time after the axoplasmic transport has completely stopped[3].

I have investigated, in the isolated nerve fibre of the untreated frog, the modifications of the action potentials following perfusion of the node of Ranvier with solutions containing vinblastin or vincristin; in this paper much faster effects are reported of both alkaloids on the properties of the membrane of the nerve fibre.

*Method.* I have studied the action of vinblastin and vincristin on the following parameters of the electric activity in isolated nerve fibres: amplitude and duration of the action potential, threshold for electrical excitability and membrane potential. Furthermore I searched for modifications of the repetitive firing which may be elicited in sensory fibres by a maintained electric stimulus[4] (about 30 msec). Single myelinated nerve fibres were isolated from the frog's sciatic nerve and transferred to a special chamber[5] where one node of Ranvier was continuously superfused. One of the neighbouring nodes was stimulated and the electric activity of the perfused node was recorded according to the air-gap technique[5]. The liquid of perfusion was a balanced salt solution for amphibia (110.5 NaCl, 2.5 KCl, 1.8 $CaCl_2$ and 2.4 $NaHCO_3$, all in mM/l) in which either vincristin sulphate or vinblastin sulphate (Eli Lilly & Company, Indianapolis, USA) could be dissolved. The fibres were stimulated by rectangular pulses of 0.2 msec with the frequency of 1 per sec.

*Results.* The amplitude of the action potential was found to be reduced by both drugs. Figure 1 shows the dose-

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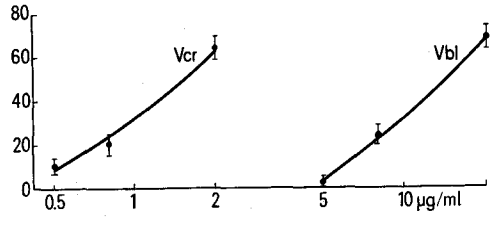


Fig. 1. Per cent decrease of the amplitude of the action potential (ordinate) at different concentrations (abscissa) of Vinblastin (Vbl) and Vincristin (Vcr).

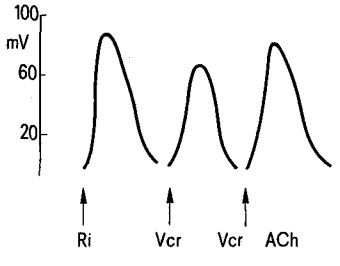


Fig. 2. Action potentials with simple Ringer (Ri), Vincristin (1µg/ml) (Vcr) and Vincristin + Acetylcholine (1 µg/ml + 1 mg/ml) (Vcr + ACh).