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Slow channel inhibitor effects on brain function: tolerance to severe hypoxia in the rat

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1 The protective effects of ten slow channel inhibitor drugs against severe progressive hypoxia were investigated in rats breathing spontaneously during light anaesthesia. Respiration, heart rate, electrocorticogram (ECoG) and/or electroencephalogram (EEG) were recorded.

2 Tolerance times were monitored from hypoxia onset until cessation of respiration, ECoG, EEG synchronization, and 'background-EEG'. Drugs were administered i.v. 5 min before the onset of hypoxia.

3 Verapamil, gallopamil, and nimodipine resulted in a significant increase of tolerance times; fendiline and bepridil showed a small increase (not significant); bencyclan and prenylamine were ineffective; cinnarizine and diltiazem slightly reduced tolerance times as did flunarizine at low doses.

4 At protective doses, verapamil, gallopamil, and nimodipine significantly raised the respiration rate but had little or no cardiac depressor effects. Bencyclan showed ventilatory drive but cardiocirculatory depression. A clear-cut ventilatory drive did not occur with the other ineffective slow channel inhibitors.

5 It is suggested that the protective actions observed were not due to slow channel inhibition *per se*, nor to spasmolytic potency or increased cerebral blood flow. Ventilatory drive associated with other cardiopulmonary actions which secondarily raise the brain oxygen supply are likely to be responsible for this effect.

Introduction

The efficacy of vasodilator drug-induced amelioration of cerebral blood flow (CBF) for treatment of impaired cerebral function due to cerebrovascular disease or cerebral metabolic disorders such as Alzheimer's disease is dubious. Rather, therapy should be focussed on controlled antihypertensive care, cardiac function support, and regulation of risk factors (Sokoloff, 1959; Gottstein, 1974; Herrschaft, 1975). Thus, rather than increasing CBF, continuing efforts are being made to establish direct therapeutic access to support brain function.

Consequently, CBF monitoring has been omitted in the present study. The test model used is based on increasing tolerance of brain function to hypoxia, i.e. postponing specific functional 'insults' that are highly sensitive to oxygen deficiency (Cartheuser, 1987). These insults are the onset of respiratory standstill and of silences in selected brain electrical activities, i.e., in ECoG 'spiking', EEG synchronization and 'background-EEG'. During standardized progressive hypoxia, these noninvasively monitored insults represent well-defined specific changes in systemic and cerebral blood gases, acid-base state, CBF, brain oxygen supply, and other invasive parameters (Cartheuser, 1987; 1988b).

Previous results have shown that some slow (calcium) channel inhibitors significantly prolonged tolerance times to cessation of respiration and brain electrical activities. Various nonspecific vasodilators and drugs interfering with cyclic AMP metabolism remained ineffective (Cartheuser, 1984a,b).

The aim of this study was to elucidate whether there are common pharmaco-physiological mechanisms by selected slow channel inhibitors that prolong cerebral hypoxic tolerance times.

Some of these results were presented at the spring

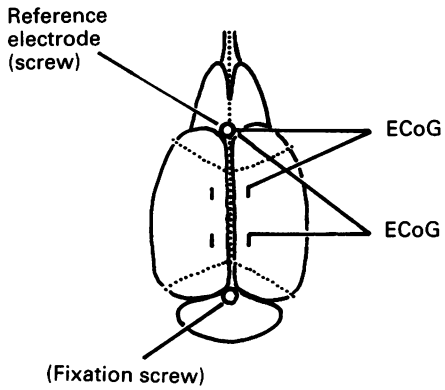

Figure 1 Localization of permanently implanted platinum epicortial electrodes and position of metallic fixation screws in rat skull. Unipolar recordings usually were aRd (anterior/reference, dextra) and pRd (posterior/reference, dextra).

meeting of the German Pharmacological Society (DPhG), Mainz, 1984, and the IUPHAR Congress, London, 1984.

Methods

Animals and implantation procedure

Four epi-cortex electrodes (platinum; 0.2 mm diameter) and two fixation screws were implanted into the skull cap of anaesthetized male albino rats (Sprague Dawley, $n = 150$; Wistar, $n = 6$; approx. 250 g) (Figure 1). The anterior screw served as a reference electrode. Along with the other electrodes it was soldered to a microsocket. The complete arrangement was embedded in Paladur resulting in a cylindrical 'crown'. The operation wound was treated with neomycin-sulphate. During the three weeks allowed for recovery the rats received standard diet (Altromin) and tap water *ad lib.*

Hypoxia experiments

Progressive hypoxia was produced in a 25 l chamber which was initially air-filled. Nitrogen was infused at constant rate (flowmeter) and the gases mixed such that O_2-concentration decreased with a half-life of 1.25 min (Cartheuser, 1987). Continuous $[O_2]$ control was achieved with a Beckman O_2 analyzer (model C), which received gas from inside the chamber at a pumping rate of approx. 150 ml per min. To ensure normobaric conditions, excess gas was allowed to escape.

'Crowned' rats received short-term anaesthesia (sodium-hexobarbitone, 130 mg kg^{-1}, i.p.) and were warmed on a thermal pillow until placed on a thermo-insulated base within the chamber (room temperature). During the initial normoxia and throughout progressive hypoxia (4 min), ECoG and/or EEG, ECG (cutaneous needle electrodes), and respiration were monitored (Schwarzer preamplifiers and multigraph). For the latter signals a thermistor attached to a bridge circuit was located close to the animal's nose.

Tolerance data recording

ECoG 'spiking' activity typical of unconscious rats decreases with increasing depth of anaesthesia. A suitable standard for tolerance time evaluation was achieved by using rats under light anaesthesia, i.e., when spike rates were above 170 per min, but with subdued nociceptive reflexes (Figure 2).

When hypoxia is severe, respiratory standstill occurs and is immediately followed by ECoG silence. Remaining background-EEG ('EEG') is then recorded, revealing increased amplitude during signal slowing, with a short period of synchronization (Syn). The Syn then ceases and is followed by a rapid decrease in amplitude and frequency until 'EEG' silence (Figure 2).

Tolerance times to silence (TTS), i.e. from start of progressive hypoxia to these subsequent cessation events (TTS-Resp; TTS-ECoG; TTS-Syn; TTS-'EEG') (Figure 2), were measured concomitant with the actual course of inspiratory oxygen concentration (CIO_2) inside the chamber. TTS-Syn occasionally occurs before TTS-ECoG in single rats, rendering mean TTS-Syn a less accurate variable for analysis. TTS-Resp and TTS-ECoG can be identified precisely and show lowest variability: variation coefficient (VC = s.d./mean) approx. 0.13; see Figures 3–5 (VC = 0.5 to 1.0 at stable hypoxia of $[O_2] = 4\%$; Cartheuser, unpublished results). Survival of animals was generally ensured by reestablishing normoxia (opening of the chamber) directly after TTS-'EEG' since, normally, brief spontaneous emergency respiration then occurs. Otherwise, immediate manual ventilation proved effective. Due to the non-invasive techniques applied, animals could repeatedly be exposed to similar trials (up to 5 times at seven-day intervals).

Statistics

Statistics were based on Student's t test for unpaired variables, i.e., each drug dose group was compared to a control group. For drug-specific comparison of dosages, ANOVA/Scheffé test was applied as indicated in figure legends. Confidence levels are given in

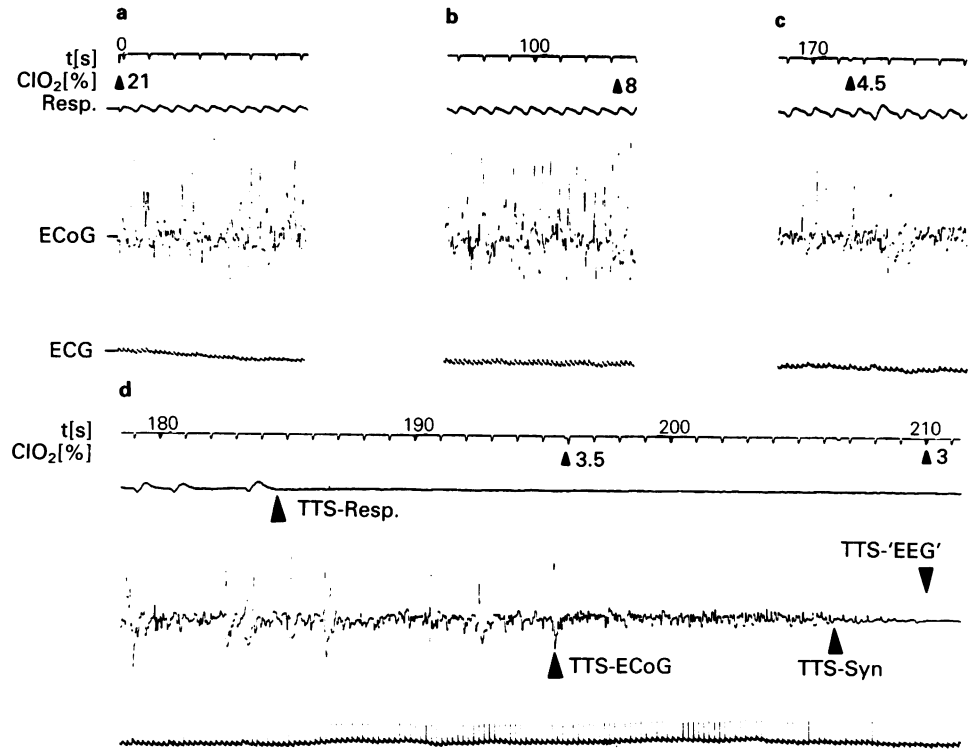

Figure 2 Sections of original recording with time scale (t[s]), inspiratory oxygen concentration (ClO_2 [%]; small arrows indicate specific values), respiration signal (Resp.), electrocorticogram (ECoG) including background 'EEG' (at $50\,\mu V\,cm^{-1}$), and electrocardiogram (ECG) of a control rat (1 ml 0.9% NaCl i.v.) during progressive hypoxia (weak hexobarbitone anaesthesia, spontaneous respiration). ECoG spike rate (calculated at half mean amplitude) rises from 210 per min (a, normoxia) to 270 per min (b), and slows down to 150 per min (c). Section (d) shows cessation of respiration (Resp.), of ECoG spiking, synchronization period (Syn), and 'EEG' at respective tolerance times to silence (TTS) as denoted by large arrows.

the figures. Values represent mean ± s.d. (or ± s.e.mean as indicated).

Drugs

Drugs were applied i.v. within 1 min, generally 5 min before starting progressive hypoxia. It was assumed that any brain specific effects for the drugs used would be manifested within this time span. Drugs tested were: bencyclan (Thiemann), flunarizine (Janssen), bepridil (Organon), gallopamil (D 600) (Knoll), cinnarizine (Janssen), nimodipine (Bayer), diltiazem (Gödecke), prenylamine (Hoechst), fendiline (Thiemann) and verapamil (Knoll).

Most drugs were prepared as aqueous solutions (prenylamine and fendiline at 40°C) from pure salts except for bencyclan (Fludilat) and verapamil (Isoptin) which were purchased as ampoules prepared by the manufacturer. Nimodipine and flunarizine were prepared in polyethyleneglycol (PEG 400) subsequently diluted in H_2O; cinnarizine and gallopamil were dissolved in 30% and 7% ethanol, respectively. Nimodipine was handled under sodium light and in light-protected syringes. Drug dosages were calculated as free base weights.

Results

Bencyclan

At doses of 1, 3 and $10\,mg\,kg^{-1}$ i.v., bencyclan failed to increase tolerance times compared to NaCl-treated control rats. Respiration rate was dose-dependently increased initially and throughout hypoxia. At $10\,mg\,kg^{-1}$, respiration rate significantly exceeded controls from the start and up to 140 s into hypoxia. Heart rate was dose-dependently reduced at 3 and $10\,mg\,kg^{-1}$, and differed significantly from controls until 140 and 200 s into hypoxia, respectively (Figure 3).

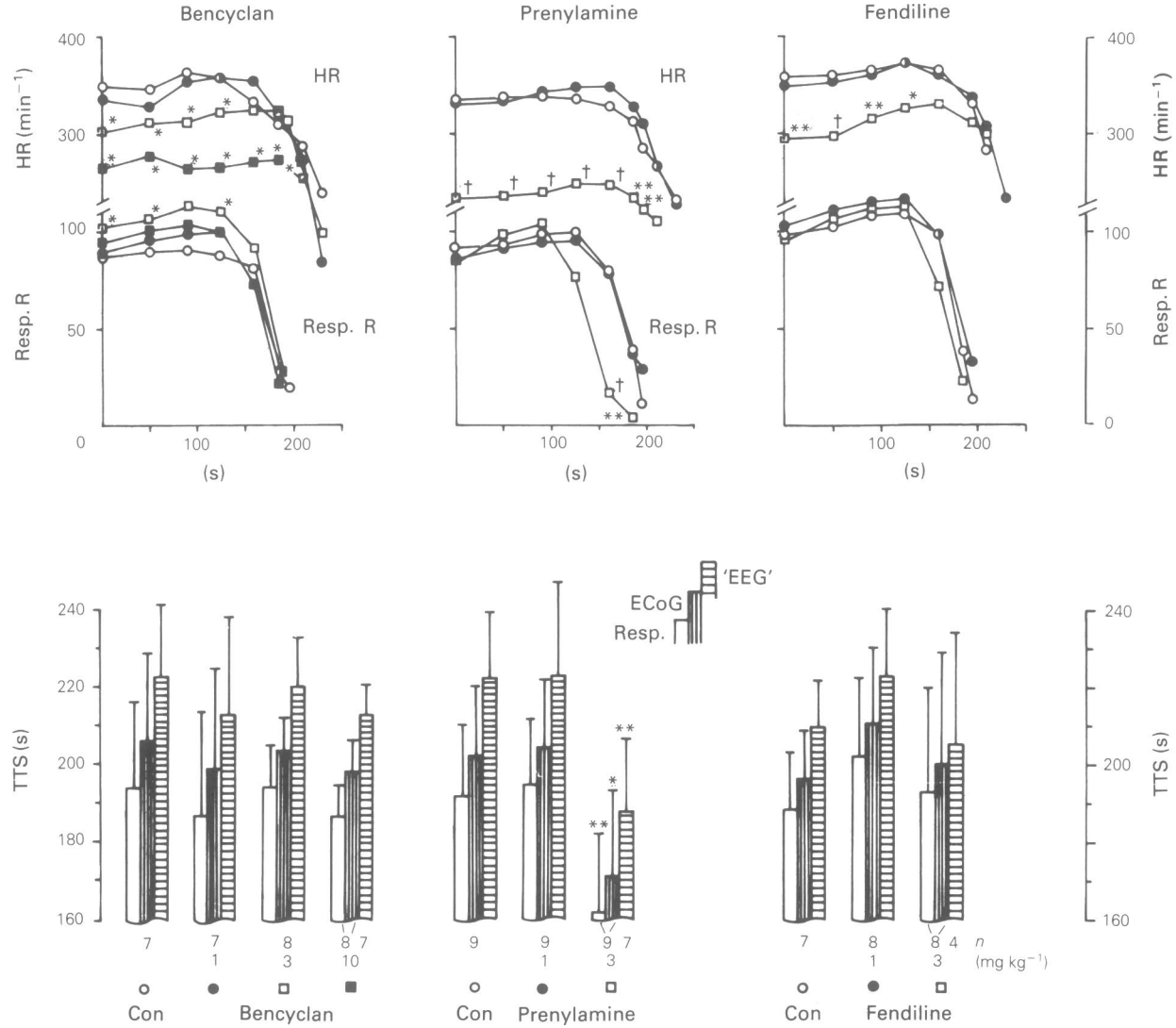

Figure 3 Effects of the nonspecific slow channel inhibitors bencyclan, prenylamine and fendiline (diphenylalkylamines) and of (drug-specific NaCl controls; Con) on rat heart rate (HR) and respiration rate (Resp.R) mean values initially at normoxia and throughout progressive hypoxia (curves, upper panels), starting hypoxia 5 min following drug administration. Columns (below) show final periods with tolerance times to silence (TTS [s]) in Resp., ECoG spiking, and background-'EEG' (mean with s.d. shown by vertical lines). Number of rats per data point indicated below the columns; body wts. (incl. respective control) were: bencyclan: 426 ± 33 g; prenylamine: 409 ± 27 g; fendiline: 372 ± 32 g. Statistics: * $P < 0.05$; ** $P < 0.01$; † $P < 0.001$ (t test vs. controls). Symbols in curves represent drug doses i.v. in 1 ml kg^{-1} each: (○) controls (0.9% NaCl); (●) 1 mg kg^{-1}; (□) 3 mg kg^{-1}; (■) 10 mg kg^{-1}.

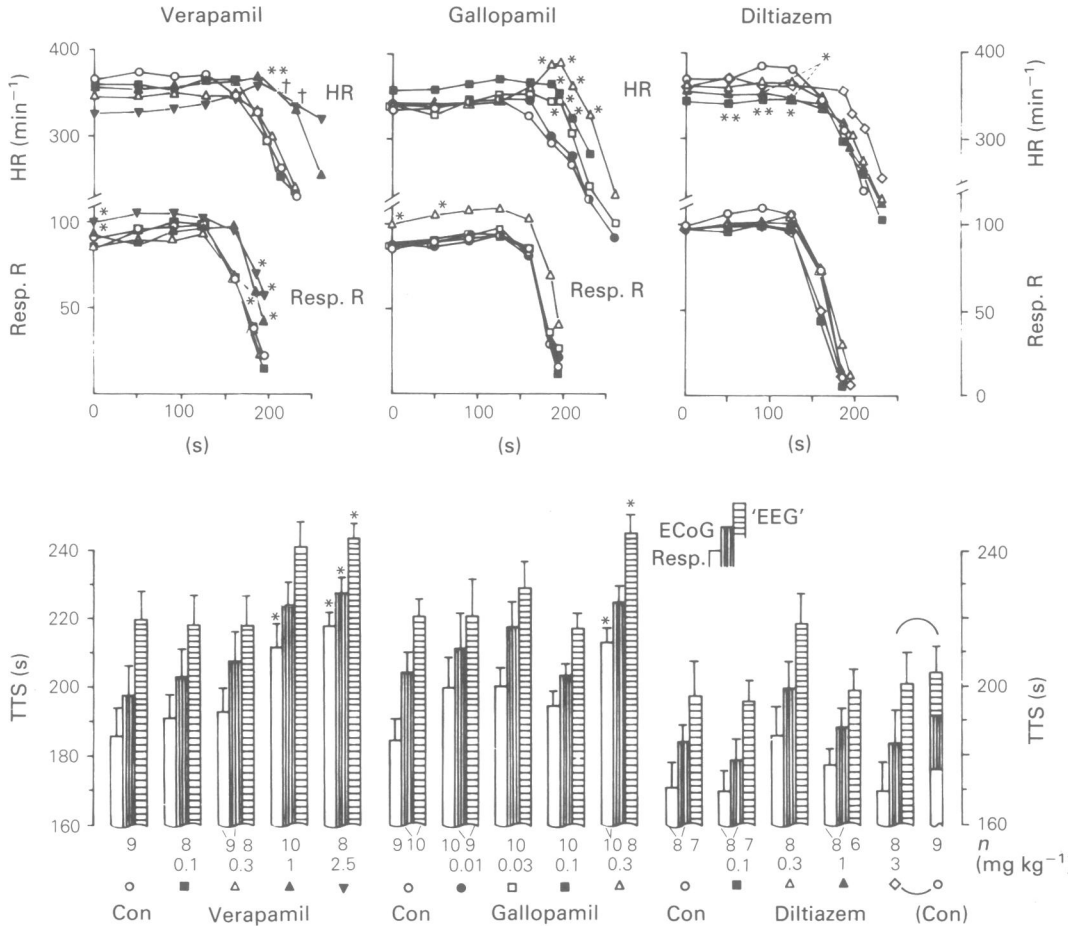

Figure 4 Comparison of the specific slow channel inhibitors (phenylalkylamines) verapamil and gallopamil, and diltiazem (benzothiazepine) versus NaCl (Con): heart and respiration rate time courses (HR, Resp R; mean values) and tolerance times to silence (TTS; columns; mean with s.e.mean shown by vertical lines) of respiration and electrical brain functions throughout progressive hypoxia in the rat. Gallopamil and diltiazem statistics: * $P < 0.05$; ** $P < 0.01$; † $P < 0.001$; t test vs. controls; verapamil was tested without a parallel control group. Statistics are therefore related to the lowest verapamil dose (ANOVA/Scheffé). For comparison, representative NaCl group (Con) data are given. Rat body wts. including respective controls were: verapamil: 429 ± 25 g; gallopamil: 430 ± 26 g; diltiazem: 382 ± 18 g. Symbols used in the HR and Resp.R. curves represent dosages i.v. in 1 ml kg^{-1} each: (○) controls (0.9% NaCl); (●) 0.01 mg kg^{-1}; (□) 0.03 mg kg^{-1}; (■) 0.1 mg kg^{-1}; (△) 0.3 mg kg^{-1}; (▲) 1 mg kg^{-1}; (▼) 2.5 mg kg^{-1}; (◇) 3 mg kg^{-1} diltiazem; tested against own control group. For further abbreviations see Figure 3.

Prenylamine

At 1 mg kg^{-1} i.v., prenylamine was ineffective but at 3 mg kg^{-1}, tolerance times were significantly reduced, as was heart rate initially and throughout hypoxia. Respiration rate nearly equalled that of controls until 90 s into hypoxia; then it fell rapidly below controls (Figure 3). A 6 mg kg^{-1} dose tended to prolong TTS-ECoG by 3% (NS; no detection of respiration signal). Prenylamine at this dose occasionally led to necroses at the injection site and was found to precipitate inside the vein. Its active dosage was thus questionable.

Fendiline

Fendiline increased tolerance times by about 7% at 1 mg kg^{-1} i.v. (NS), revealing no effect on heart rate but a slight tachypnoea of about 10% (NS). At 3 mg kg^{-1} i.v., tolerance times and respiration rate

were similar to controls, but heart rate was strongly depressed initially and throughout hypoxia ($P < 0.01$; Figure 3). In a study with only TTS-ECoG evaluation in Wistar rats, 6 mg kg^{-1} induced a slight (6%) 'increase' (NS) of this variable.

Verapamil

Verapamil (0.1, 0.3, 1, 2.5 mg kg^{-1}, i.v.) produced a dose-dependent increase of tolerance times (Figure 4). At 1 mg kg^{-1} only respiration and synchronization tolerance times were significantly prolonged. At 2.5 mg kg^{-1}, TTS-Resp, TTS-Syn and TTS-'EEG' were significantly increased (TTS-Syn is a less accurate parameter).

Heart rate was reduced slightly in a dose-dependent way. With the higher doses, heart rate remained constant for a longer period so that in the late stages of hypoxia it was significantly higher than after 0.1 mg kg^{-1}. Respiration rate was initially increased at 1 and 2.5 mg kg^{-1} and also remained stable for a longer period than with the lowest dose (Figure 4).

Gallopamil (D 600)

Gallopamil (0.01, 0.03, 0.1, 0.3 mg kg^{-1}, i.v.) produced dose-dependent increases in respiratory and 'EEG' tolerance times (except at 0.1 mg kg^{-1}), differing significantly from the respective controls at the 0.3 mg kg^{-1} dose. With 0.01 mg kg^{-1}, heart rate changes were similar to controls. At both the intermediate doses heart rate remained constant for a longer period (200 s). With 0.3 mg kg^{-1}, at 150 s into hypoxia, heart rate further increased and was significantly higher compared with controls and with the 0.01 mg kg^{-1} dose. Respiration rate did not differ from controls except at the 0.3 mg kg^{-1} dose, which initially provoked significant tachypnoea (Figure 4).

Diltiazem

This drug (0.1, 0.3, 1, 3, 9 mg kg^{-1}, i.v.) did not change tolerance times at the lower doses except for a slight increase (NS) at 0.3 mg kg^{-1} (Figure 4) but dose-dependently reduced tolerance times (NS) at 1, 3 and 9 mg kg^{-1}. The 9 mg kg^{-1} dose led to immediate death in two rats, and tended to reduce tolerance times in the remaining rats. The tendency of diltiazem to reduce tolerance times (NS) was also confirmed in Wistar rats at a 3 mg kg^{-1} dose. At most doses the respiration rate was slightly below controls (NS). Heart rate was reduced most with 0.1 mg kg^{-1} and approached control levels at higher doses (except at 1 mg kg^{-1} in Figure 4).

Cinnarizine

Cinnarizine (1, 3 mg kg^{-1}, i.v.) slightly reduced tolerance time variables (NS). Heart rate was unaffected even at the upper dose, and respiration rates equalled those of respective controls.

Flunarizine

Flunarizine at 0.03, 0.1 and 0.3 mg kg^{-1}, i.v., dose-dependently reduced tolerance times (NS). The 0.1 mg kg^{-1} dose caused a significant reduction of maintenance of the 'EEG'. Only at 1 mg kg^{-1} did tolerance times to respiratory standstill and ECoG silence slightly (NS) exceed control values; differences were significant in respect to the 0.1 mg kg^{-1} dose ($P < 0.05$). Flunarizine dose-dependently reduced heart rate compared to controls, an effect that was significant from 140 s hypoxia with 0.3 mg kg^{-1}. At 1 mg kg^{-1}, strong bradycardia persisted initially and up to 160 s into hypoxia. Respiration rate was (NS) variably altered at most doses; transient acceleration ($P < 0.05$) with the 0.3 mg kg^{-1} dose was observed (Figure 5).

Nimodopine

Nimodipine at 0.1, 0.3, 1 and 2 mg kg^{-1}, i.v., dose-dependently raised tolerance times. This was demonstrated by significant elevations of TTS-Syn and TTS-Resp at 1 and 2 mg kg^{-1}. Heart rate was slightly (NS) reduced with increasing doses. Respiration was accelerated from 0.3 mg kg^{-1}, being significant with the 2 mg kg^{-1} dose initially and up to 140 s into progressive hypoxia (Figure 5).

Bepridil

Bepridil (0.1, 0.3, 1, 3 mg kg^{-1} i.v.) dose-dependently increased tolerance times (NS) by up to 10%. Heart rate was dose-dependently reduced ($P < 0.05$ at 1 and 3 mg kg^{-1}), and respiration was slightly accelerated (NS), initially and up to 150 s into hypoxia (Figure 5).

Discussion

Though the model used in this study is merely one of controlled 'cerebral salvage', it has several advantages in that standardized progressive hypoxia provides a specific time course of changes in many physiological parameters (Cartheuser, 1987). Hence, focussing on cessation of functions (apnoea and brain electrical silences) provides a marker for well-

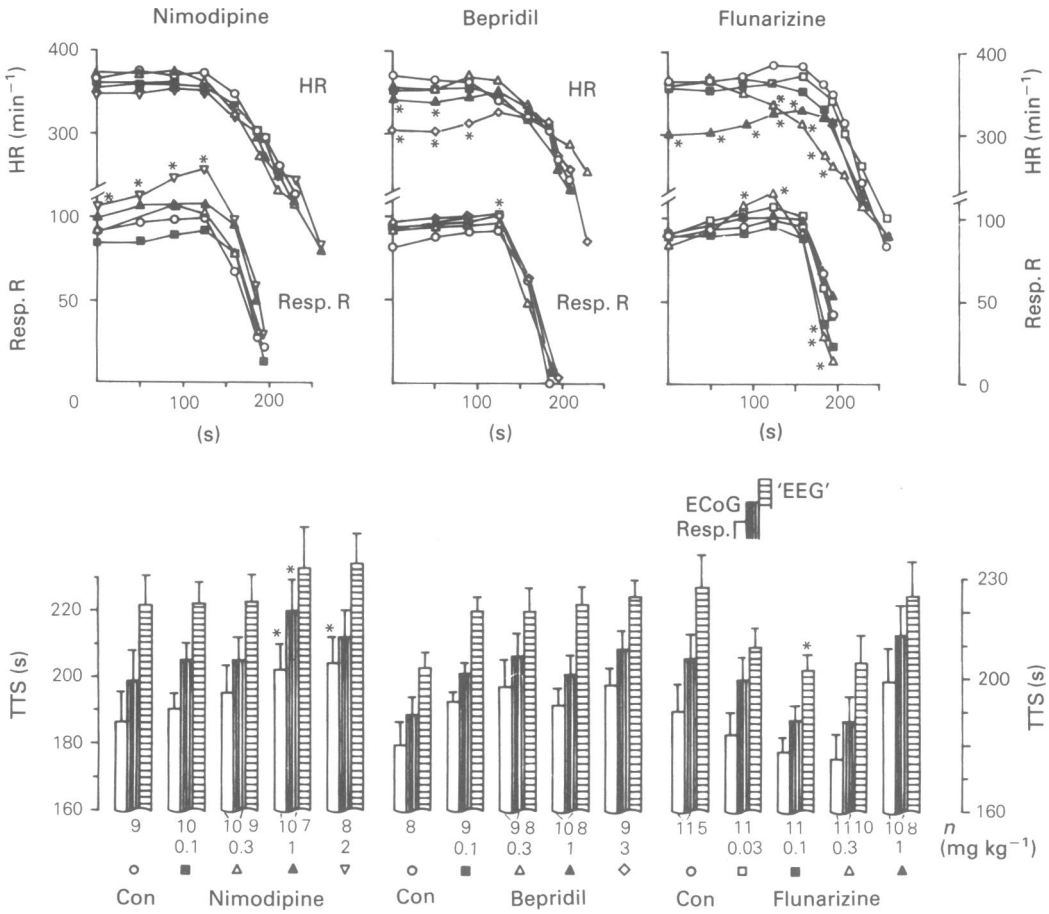

Figure 5 Comparison of effects by the specific slow channel inhibitors nimodipine (dihydropyridine), bepridil and flunarizine (diphenylbutylpiperazine) versus NaCl (Con) throughout progressive hypoxia: rat heart and respiration rate time courses (HR, Resp. R; mean values) and (columns) tolerance times to silence (TTS; mean with s.e.mean shown by vertical lines) of respiration and electrical brain functions. Rat body wts. including respective controls were: nimodipine: 403 ± 32 g; bepridil: 407 ± 26 g; flunarizine: 426 ± 25 g. Statistics: * $P < 0.05$; ** $P < 0.01$; † $P < 0.001$ (t test vs. controls; flunarizine: ANOVA/Scheffé). Symbols used in the HR and Resp.R. curves represent dosages i.v. in 1 ml kg^{-1} each: (○) controls (0.9% NaCl); (□) 0.03 mg kg^{-1}; (■) 0.1 mg kg^{-1}; (△) 0.3 mg kg^{-1}; (▲) 1 mg kg^{-1}; (▽) 2 mg kg^{-1} (nimodipine at 3 ml kg^{-1}); (◇) 3 mg kg^{-1}. Further abbreviations see Figure 3.

defined 'insults' (Cartheuser 1988b). In control rats these insults occur when arterial Pco_2 and pH, having been shifted to alkalosis by prior hyperpnoea, re-attain normal values (Cartheuser, 1987). Therefore, brain electrical activities and CBF, which closely depend on arterial Pco_2 and pH (Bremer & Thomas, 1936; Lennox *et al.*, 1938; Gibbs *et al.*, 1940; Lassen, 1968; Alberti *et al.*, 1975), are then governed primarily by the lowered arterial Po_2 (Gibbs *et al.*, 1940; Kogure *et al.*, 1970). At the time of cessation of functions, however, CBF elevation is poor (26 to 59% of initial) (Cartheuser, 1987) when compared to the possible maximum of almost five-fold of normal flow (Eklöf *et al.*, 1973; Bergström *et al.*, 1975). An increase of CBF beyond changes in control rats should thus be possible throughout progressive hypoxia with any real vasodilator.

Certain vasodilators, claimed to increase CBF in animals or men, e.g. papaverine, vincamine, methylxanthines, meclofenoxate (Sokoloff, 1959; Komarek *et al.*, 1977; Hutten *et al.*, 1980), however, did not increase tolerance times in this model (Cartheuser,

1984b). The only exception was an infusion of nicotinic acid (see below). For several reasons, protection cannot be due to general CBF elevation since: (1) cerebral vasodilators in general failed to increase hypoxia tolerance (Cartheuser, 1984b); (2) ventilatory drive (hyperventilation), typical for all drugs effective in the present model, reduces CBF (Lennox *et al.*, 1938; Alberti *et al.*, 1975); (3) verapamil exerted its protection against hypoxia without CBF-elevation beyond controls (Cartheuser, 1985; Cartheuser, 1987); and (4) verapamil and nimodipine did not increase local CBF at focal cerebral ischaemia in rats (Gotoh *et al.*, 1986; Hakim, 1986).

Hypoxia-induced pulmonary vasoconstriction (Von Euler & Liljestrand, 1946; Atwell *et al.*, 1954; McMurtry *et al.*, 1982) is suggested to be critically dependent on transmembrane Ca^{2+} influx: verapamil and the slow channel inhibitor SKF 525A depressed this pressor response (McMurtry *et al.*, 1976). Slow channel inhibitors relax experimentally-induced spasms in cerebral, pial and basilar arteries (Kamiya, 1980; Edvinsson *et al.*, 1980; 1983; White *et al.*, 1982; Müller-Schweinitzer & Neumann, 1983) and CBF may be ameliorated (Herrschaft, 1976; Harper *et al.*, 1981; Kazda *et al.*, 1982; Mohamed *et al.*, 1983). However, it can neither be assumed that brain protection in the model used was due to Ca^{2+} channel inhibition *per se* nor that the protection with verapamil, gallopamil and nimodipine (Figures 4, 5) resulted from a spasmolytic action: cerebral vasospasms are not likely to occur in intact rats and most representatives of the Ca^{2+} inhibitors failed to be protective.

Verapamil increased cerebral oxygen supply during severe hypoxia thereby prolonging cerebral electrical functions (Cartheuser, 1987). Better oxygen supply than in controls was achieved by stronger arterial alkalosis allowing higher blood oxygen saturation, and by stronger cerebral venous acidosis allowing higher O_2 desaturation of blood in the brain; arterial alkalosis was secondary to significant hyperventilation (Cartheuser, 1985; Cartheuser, 1987). Out of numerous slow channel inhibitors and other drugs tested in the present model, a significant hyperpnoea has only been obtained with verapamil, gallopamil, nimodipine, and bencyclan (this study), and with vasopressin and nicotinic acid, both as infusions (Cartheuser, 1984a,b; 1986). Except for bencyclan, only these drugs significantly prolonged tolerance times. These results suggest that hyperpnoea (ventilatory drive) is the common drug effect primarily responsible for brain protection against hypoxia.

In contrast to the protective agents verapamil, gallopamil and nimodipine which, from 5 min after injection, exerted no or only weak effects on heart rate, bencyclan produced a significant dose-dependent bradycardia (Figure 3). A reduced cardiac oxygen demand occurring with bradycardia may be expected to result in a higher oxygen availability for the brain. However, tolerance times were impaired with severe bradycardia. This was presumably due to cardiodepression and severe hypotension and, hence, a detrimental reduction of cerebral perfusion. Considering bencyclan, cardiac and circulatory depression (Köhler *et al.*, 1975) may have prevented a presumptive cerebral protection via its ventilatory drive. Similar deleterious bradycardia also occurred with fendiline, flunarizine and bepridil all tending to tachypnoea (Figures 3, 5), and by lower doses of diltiazem (Figure 4).

With flunarizine, the dose-dependent decrease in hypoxia tolerance associated with bradycardia was reversed with $1\,mg\,kg^{-1}$ (Figure 5) even though the bradycardia was further accentuated. This suggests a beneficial central action at this higher dose. Whether this might be a protection against neuronal calcium overload (Clincke & Wauquier, 1982) seems unlikely since such a calcium overload occurs only during longer time spans of anoxia (Krieglstein & Weber, 1986).

Bradypnoea (ventilatory depression), associated with bradycardia, additionally reduces tolerance times as demonstrated with prenylamine ($3\,mg\,kg^{-1}$; Figure 3).

No significant effects on heart and respiration rates were obtained with higher doses of diltiazem (Figure 4) or with cinnarizine, although they decreased tolerance times. With cinnarizine, this may have occurred due to sedation and anti-excitatory actions related to its antihistaminic properties as seen with flunarizine (Ell & Gresty, 1983). With diltiazem, hypotension may have impaired the hypoxia tolerance. Bradycardia occurring only with the lower doses may be explained by heart rate 'reflex stabilization' developing with severely lowered blood pressure induced by high doses of diltiazem (Bourassa *et al.*, 1980). Hyperventilation (ventilatory drive) might be considered a component of CNS excitation. CNS excitation independent of ventilatory drive is not likely to extend cerebro-electric function during progressive hypoxia: the CNS excitatory drugs pentylenetetrazol and pyridoxine (Goodman & Gilman, 1966) failed to prolong and the latter even decreased, tolerance times (Cartheuser, unpublished results). Verapamil depressed cerebral epileptic seizures (Walden *et al.*, 1985) and, in the present study, did not alter spontaneous ECoG activity at normoxia or mild hypoxia; it slightly increased ECoG spike rate (Figure 6) beyond that of control rats only at medium and severe hypoxia. Respiration, however, was significantly accelerated initially at normoxia.

The finding that cerebral venous pH shifts to acidosis with verapamil (Cartheuser, 1985) may indicate

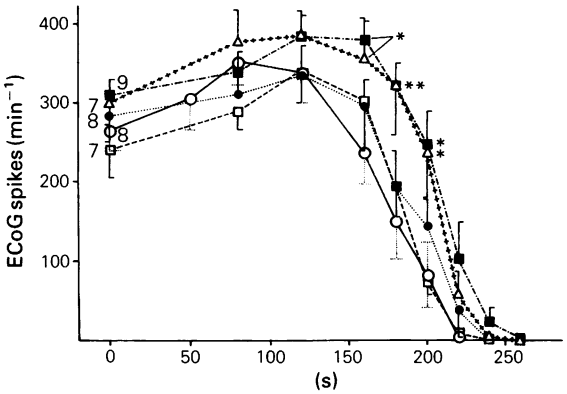

Figure 6 Courses of ECoG spike activities in anaesthetized rats throughout progressive hypoxia: effects of verapamil at doses of 0.1 (●); 0.3 (□); 1.0 (■) and 2.5 (△) mg kg^{-1} i.v., compared to controls (0.9% NaCl i.v.); 1 ml kg^{-1} each. Number of animals per group indicated; values are mean with s.e.mean shown by vertical lines; * $P < 0.05$ vs controls.

either higher cerebral metabolic activity or a higher proton efflux from hypoxic brain cells (Cartheuser, 1987). Gallopamil infusion during post-ischaemic reperfusion in isolated brains decreased cerebral lactate levels faster than in controls (Krieglstein & Weber, 1986), and verapamil and nimodipine prevented cerebral tissue acidosis at focal ischaemia (Hakim, 1986). These findings may be indicative for an improved ability of the ischaemic cells to discharge their proton load (Hakim 1986; Cartheuser, 1987). The underlying mechanism may be Ca^{2+} channel inhibition assumed to induce complex ionic exchanges across the cell membrane, resulting in higher $[H^+]$ fluxes from hypoxic cells (for references: Hakim, 1986). This, necessarily, implies a brain-specific drug action, i.e., drugs must be able to pass through the blood-brain-barrier (BBB). This, however, is doubtful for several slow channel inhibitors (Edvinsson *et al.*, 1983) including verapamil (Cartheuser, 1987), at least for the intact BBB.

Brain-specific antihypoxic drugs should increase the time spans from apnoea to the subsequent cessation of brain functions. However, a clear-cut increase of these intervals was never observed in previous studies (Cartheuser, 1984a,b) nor in the present progressive hypoxia studies. Tolerance times were typically shifted 'en bloc' (except for 'Syn' due to scatter). A brain-specific effect by verapamil, gallopamil and nimodipine may also be excluded due to the lack of protection in anoxia and decapitation trials (Cartheuser, 1987; Cartheuser, 1988a).

It may thus be inferred that function tolerance prolongation in this study is primarily related to maintained respiration and to an adequate cerebral perfusion pressure. The latter does not necessarily imply CBF elevation (Cartheuser, 1987). A prolonged function during hypoxia is subject to multifactorial influences, i.e. by an extended stabilization of haemodynamics and by distinct changes in acid-base balance and gas transport mechanisms. Cardiac protection during hypoxia, e.g. with verapamil and gallopamil (Fleckenstein-Grün *et al.*, 1984) and also in the present study (Figure 4), undoubtedly represents an important factor.

The chemically heterogeneous slow channel inhibitors are known to exhibit divergent profiles of action (Zelis & Flaim, 1981; Edvinsson *et al.*, 1983; Fleckenstein-Grün *et al.*, 1984). It is concluded that only some of these drugs, besides causing ventilatory drive, provide the specific actions which together produce their protective efficacy during hypoxia.

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