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K^+ Concentration influences methohexitone inhibition of K^+-stimulated release of [^{3}H]noradrenaline from minislices of rat cerebral cortex

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Abstract—This study investigated the effect of methohexitone, a barbiturate anaesthetic, with variation of the K^+ concentration used to stimulate [^{3}H]noradrenaline release from superfused minislices of rat cerebral cortex. The amount of [^{3}H]noradrenaline released by K^+-stimulation depended on the concentration of K^+ used (increasing release was seen at 10–50 mM K^+). Methohexitone (10^{-7}–3×10^{-4} M) added to the superfusing medium did not alter the basal efflux of [^{3}H]noradrenaline, but high concentrations inhibited K^+-stimulated release. Using 12·5 mM K^+, inhibition of release was observed only at 10^{-4} M methohexitone (48% inhibition); the effect of 25 mM K^+ was reduced by 3×10^{-5} M (72% inhibition) and higher concentrations while release stimulated by 50 mM K^+ was inhibited by 10^{-5} M methohexitone (30% inhibition) and higher concentrations.

Noradrenaline is widely but fairly diffusely distributed throughout the cerebral cortex. It can be released from cortical tissue preparations by depolarization of the tissue in a variety of ways, including the use of raised extracellular potassium concentrations, veratridine or electrical stimulation (Blaustein et al 1972; Vargas & Orrego 1976; Taube et al 1977). The amount of noradrenaline released from the tissue preparation depends on the magnitude of the K^+ stimulus used (Vargas & Orrego 1976).

Various authors have shown that the barbiturate anaesthetics can inhibit the K^+ stimulated release of noradrenaline from such preparations (de Boer et al 1982; Jones & Symington 1990). There is considerable variation in the magnitude of the effects observed. It was thought that this might, at least in part, be related to the stimulus intensity. The present study investigated whether the effect of methohexitone, a barbiturate anaesthetic, would vary with the K^+ concentration used to stimulate [^{3}H]noradrenaline release from superfused minislices of rat cerebral cortex.

Materials and methods

Methods. Male Wistar rats, 120–150 g, were killed by stunning and cervical dislocation. The brains were rapidly dissected and placed on an ice-cold surface and the pia removed. The cerebral cortex was separated, and chopped on a McIlwain tissue chopper at 350 μm intervals, in two directions. The minislices were suspended in 4 mL modified Krebs-phosphate buffer (composition (in mM): NaCl 118, KCl 4·8, NaH_2PO_4 15, $CaCl_2$ 1·2, $MgSO_4$ 1·2, D-glucose 5·6, pargyline 0·01), then allowed to settle. The supernatant (containing cell debris) was discarded and the minislices transferred to an incubation flask containing 8 mL buffer. The minislices were incubated at 37°C for 10 min under an atmosphere of O_2 in a shaking water bath. [7,8-^{3}H]Noradrenaline (185 kBq, sp. act. 444 GBq mmol^{-1}, final concentration 46 nmol L^{-1}) was added and incubation continued for a further 15 min. The supernatant was removed, and the tissue divided amongst eight superfusion chambers where it was held between nylon mesh grids, and superfused with Krebs-phosphate buffer (including desipramine 0·1 μM) at 37°C at 500 μL min^{-1} per chamber.

After a 30 min equilibration period (during which the superfusate was discarded), 2 min fractions of superfusate were collected for the following 46 min (the experimental period).

After 6 and 30 min of the experimental period the superfusing medium was changed for 2 min to one containing elevated K^+. Where appropriate, at 18 min the solution was changed to one containing methohexitone, and this was then maintained for the rest of the experiment. At the end of the experimental period the tissue was removed from each chamber, lysed in 10 mL water, and an aliquot taken for estimation of ^{3}H, to allow calculation of the total radioactivity remaining in the tissue.

The ^{3}H content of the samples was estimated by liquid scintillation counting and this was assumed to reflect [^{3}H]noradrenaline efflux from the tissue. Results were calculated as the fraction of total tissue radioactivity released during each 2 min collection period. Basal release (B_1, B_2) was taken to be the mean release during the 4 min immediately preceding each pulse of elevated K^+. Stimulated release (S_1, S_2) was calculated as the increase in release over basal in the 10 min during and immediately after each pulse of high K^+. The S_2/S_1 ratio was used to assess the effects of methohexitone treatment.

In experiments to determine Ca^{2+}-dependence of the release, Ca^{2+} was omitted from the Krebs buffer, Mg^{2+} concentration was increased to 10 mM, and NaCl was reduced to maintain osmolarity. Where elevated K^+ concentrations were used, Na^+ concentration was reduced.

HPLC analysis using electrochemical detection (Reynolds 1983) confirmed that >90% of the ^{3}H efflux, under either basal or K^+-stimulated conditions, co-eluted with noradrenaline (data not shown).

All results are expressed as mean ± s.e.m. Statistical significance was determined by analysis of variance and *t*-test, or paired *t*-test, as appropriate.

Materials. Pargyline and desipramine were purchased from Sigma Chemical Co Ltd, Poole, Dorset, UK; methohexitone from Eli Lilly & Co. and [7,8-^{3}H]noradrenaline from Amersham International, Aylesbury, Bucks, UK. All other materials used were of analytical reagent grade.

Results

Basal efflux. The mean basal efflux of [^{3}H]noradrenaline from minislices of rat cerebral cortex was 0·73 ± 0·03% of the total tissue content per 2 min collection period initially (B_1) and 0·60 ± 0·02% before the second pulse of high K^+ (B_2). Values are mean ± s.e.m. for 144 channels. The B_2 values were slightly, but significantly, lower than B_1 values ($P < 0·001$, paired *t*-test).

In the absence of Ca^{2+}, basal efflux was 60 ± 3·5% lower (n = 16) than concurrent control channels superfused with Krebs-phosphate buffer. In the absence of exposure to elevated K^+ concentrations the efflux of [^{3}H]noradrenaline remained virtually unchanged during the experimental period (Fig. 1).

K^+-Stimulated release. Changing the superfusing medium for 2 min to one containing an elevated K^+ concentration resulted in an increase in [^{3}H]noradrenaline release during the following 10 min period. Exposure to a second pulse of high K^+ 24 min after

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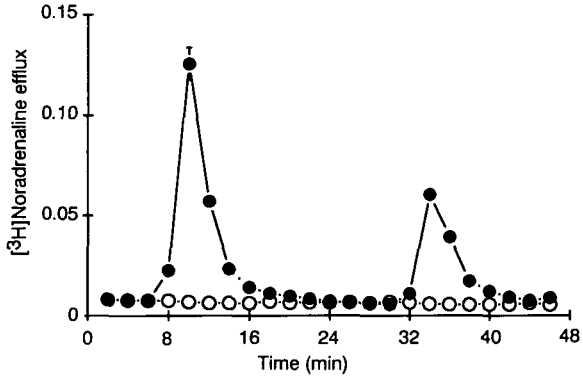

FIG. 1. [^{3}H]Noradrenaline efflux from minislices of rat cerebral cortex stimulated by exposure to 2 min pulses of high K^+ Krebs buffer (at 6 and 30 min). Efflux is expressed as a fraction of the total tissue [^{3}H]noradrenaline released in each 2 min period. Points are means (most s.e.m. are smaller than symbols). ● 25 mM K^+ pulse, n = 46; ○ unstimulated, n = 13.

the first exposure resulted in an increase in release over basal efflux which was consistently smaller than the first response (Fig. 1).

This response to elevated K^+ concentration was very much attenuated in the absence of Ca^{2+} (12·5 mM K^+ 2·4% control, 25 mM K^+ < 1% control, 50 mM K^+ 10% control), confirming that the K^+-stimulated release of [^{3}H]noradrenaline from minislices of rat cerebral cortex is Ca^{2+}-dependent.

The amount of [^{3}H]noradrenaline released by K^+-stimulation was found to depend on the concentration of K^+ used. Results are summarized in Table 1. More [^{3}H]noradrenaline is released by high concentrations of K^+ than by lower concentrations. However, the ratio of the amount of [^{3}H]noradrenaline released by the two pulses of K^+ remained approximately constant ($S_2/S_1 = 0·5$) for K^+ concentrations from 10 to 40 mM. At 50 mM K^+, S_2/S_1 was found to be $0·72 \pm 0·03$ (n = 31) (Table 1).

Methohexitone. Addition of methohexitone (10^{-7}–3×10^{-4} M) to the superfusing medium did not alter the basal efflux of [^{3}H]noradrenaline when compared with the appropriate control channels (e.g. 3×10^{-4} M methohexitone $B_2 = 0·0057 \pm 0·0004$ (n = 15) vs controls $B_2 = 0·0055 \pm 0·0004$ (n = 43)).

Methohexitone did inhibit K^+-stimulated release of [^{3}H]noradrenaline in a concentration-dependent manner (Table 2). Using 25 mM K^+, concentrations of methohexitone of 3×10^{-5} M or higher resulted in a S_2/S_1 ratio significantly lower than that obtained for drug-free controls. Lower concentrations did not significantly alter the ratio.

When the effect of methohexitone was studied using K^+ pulses of 12·5 or 50 mM, different concentration-response relationships were obtained from those observed with 25 mM K^+. A significant inhibition of K^+-stimulated [^{3}H]noradrenaline release was observed only at 10^{-4} M methohexitone when 12·5 mM K^+ pulses were used, but with 50 mM K^+, all concentrations of methohexitone studied (10^{-5}–10^{-4} M) caused a significant inhibition of release. The response to any given concentration of methohexitone appeared to be greater with 50 mM K^+, than with 25 or 12·5 mM K^+. For example, compared with methohexitone-free controls, 3×10^{-5} M methohexitone inhibited release by 12, 28 and 31% respectively for 12·5, 25 and 50 mM K^+.

Table 1. K^+-Stimulated [^{3}H]noradrenaline release and K^+ concentration.

K^+ (mM)	n	S_1	S_2	S_1/S_2
4·8	13	$-0·005 \pm 0·001$	$-0·004 \pm 0·001$	NA
10	7	$0·025 \pm 0·004$	$0·012 \pm 0·003$	$0·50 \pm 0·08$
12·5	39	$0·084 \pm 0·006$	$0·043 \pm 0·004$	$0·52 \pm 0·03$
15	7	$0·130 \pm 0·016$	$0·058 \pm 0·011$	$0·44 \pm 0·06$
25	43	$0·205 \pm 0·011$	$0·106 \pm 0·007$	$0·52 \pm 0·02$
40	4	$0·249 \pm 0·013$	$0·137 \pm 0·012$	$0·55 \pm 0·03$
50	31	$0·325 \pm 0·025$	$0·236 \pm 0·022$	$0·72 \pm 0·03$

S_1 and S_2 represent the increase in [^{3}H]noradrenaline release over basal release stimulated by a 2 min pulse of high K^+ Krebs buffer. All values are mean ± s.e.m. NA = not applicable.

Table 2. Effect of K^+ concentration on response to methohexitone.

Methohexitone (M)	K^+ (mM) 12·5	25	50
0	$0·52 \pm 0·03$ (39)	$0·52 \pm 0·02$ (43)	$0·72 \pm 0·03$ (31)
10^{-5}	$0·49 \pm 0·05$ (12)	$0·49 \pm 0·03$ (16)	$0·51 \pm 0·04$* (9)
3×10^{-5}	$0·46 \pm 0·05$ (19)	$0·38 \pm 0·02$** (27)	$0·50 \pm 0·04$* (9)
10^{-4}	$0·27 \pm 0·04$** (13)	$0·22 \pm 0·02$** (16)	$0·31 \pm 0·04$** (7)

Values are S_2/S_1 ratio, mean ± s.e.m. (number of replicates). *$P < 0·01$, **$P < 0·001$, Student's t-test, methohexitone vs control.

Discussion

Previous work from this laboratory has shown there to be a concentration-dependent inhibition of noradrenaline release from rat cortical tissue in the presence of pentobarbitone, thiopentone or methohexitone (Jones & Symington 1990). The present results show that the methohexitone effect increases as the extracellular K^+ concentration is increased. This occurs at barbiturate concentrations close to the estimated anaesthetic concentration of 19 μM (Minchin 1981).

Previous reports of studies of anaesthetic barbiturate effects in-vitro show marked variability. Baldessarini & Kopin (1967) reported that thiopentone caused a small inhibition of electrically-stimulated release of noradrenaline from brain slices, but

this did not reach statistical significance. In studies on electrically stimulated release of noradrenaline from rat cortical tissue, Lidbrink & Farnebo (1973) found pentobarbitone (10^{-4}–10^{-6} M) had no effect whereas Carmichael & Israel (1975) showed that 10^{-3} M pentobarbitone inhibited [^{3}H]noradrenaline release. Pentobarbitone (50–1000 μM) also inhibited [^{3}H]noradrenaline release stimulated by 30 mM KCl (de Boer et al 1982). Fung & Fillenz (1984) showed that in rat hippocampal synaptosomes release of noradrenaline stimulated by 25 mM K^+ was inhibited by 10^{-4} and 10^{-5} M pentobarbitone. Jones & Symington (1990) reported inhibition of noradrenaline release stimulated by 25 mM K^+ by methohexitone (10^{-5} M, 10^{-4} M and higher), pentobarbitone (3×10^{-4} M and higher) and thiopentone (3×10^{-5} M and higher).

Some differences are to be expected in the studies reported as a variety of stimulation parameters was used, and there were differences in the source and handling of the tissue (rat, guinea-pig; brain, cortex, hippocampus; slices, minislices, synaptosomes; superfusion or batch process). However, differences in the degree of depolarization induced would also contribute to the effects observed. Where elevated extracellular K^+ concentration was used as a stimulus, the concentration of K^+ and length of exposure must be considered. The flow and mixing characteristics of each apparatus will determine the exact concentration of K^+ achieved at the tissue surface (and that may not be constant throughout the chamber). Size and shape of the tissue fragments will also determine penetration of K^+ to the presynaptic terminal membrane (Nicholson & Hounsgaard 1983). Therefore, some of the variation in results reported may be due to differences in effective exposure to K^+.

It still remains unclear why differences in K^+ exposure should alter the effect of methohexitone. It is known that depolarization of brain tissue opens voltage-gated Ca^{2+} channels and the resulting influx of Ca^{2+} brings about release of neurotransmitter (Cotman et al 1976). The precise membrane potential achieved (and various other factors) will determine the exact population of ion channels opened. Barbiturate anaesthetics are thought to inhibit neurotransmitter release by decreasing Ca^{2+} entry into the terminal in response to depolarization (Willow & Johnston 1983). Gundersen et al (1988) showed that various barbiturates decreased Ca^{2+} currents flowing through Ca^{2+} channels. There is also evidence that they may increase the conductances of Cl^- (Willow & Johnston 1983) and K^+ (Nicoll & Madison 1982) and so hyperpolarize the cell. High concentrations of some anaesthetics may alter the resting intracellular free Ca^{2+} concentration (Daniell & Harris 1988) but this has not been reported for the barbiturates. These actions may in turn lead to further changes in membrane conductance. The changes in noradrenaline release observed may be due to the combination of these actions with the different degrees of depolarization induced by different concentrations of K^+.

A further factor to be considered is the existence of more than one intracellular storage pool of noradrenaline. There is evidence for at least 2 pools of noradrenaline in the presynaptic terminal (Fillenz 1990): a readily releasable pool which contains newly synthesized noradrenaline, and a less easily released pool. It is possible that [^{3}H]noradrenaline may not label these pools equally; there is clear evidence that this applies to dopamine (Herdon et al 1985). Different degrees of depolarization may release different proportions from these two pools. The pools may show different sensitivity to methohexitone effects. If methohexitone is most effective at inhibiting release from the less accessible pool, then this may account for the greater effect at 50 mM K^+ than at 12·5 mM K^+ (assuming that lower concentrations of K^+ release mainly from the most accessible pool, and that higher concentrations release increasing proportions from the less accessible pool). Therefore different pools may release to different extents depending on the K^+ concentration. The effect of methohexitone may be determined, at least in part, by the pool from which noradrenaline is released.

The effects reported here for methohexitone are in marked contrast to the effects of halothane, a volatile anaesthetic, reported by Bazil & Minneman (1989), who found that halothane inhibited K^+-stimulated noradrenaline release, but increasing the K^+ stimulus decreased the effect. This is consistent with the report of Daniell & Harris (1988) of a difference between halothane and a barbiturate anaesthetic in changes in intracellular Ca^{2+}. They found that preincubation of synaptosomes with anaesthetic concentrations of pentobarbitone inhibited the K^+-stimulated increase in intracellular Ca^{2+}, whereas preincubation with halothane markedly increased the response, but only at concentrations higher than those required for anaesthesia.

Similarly, Richter & Waller (1977) studied the effect of pentobarbitone on the release of acetylcholine from superfused minislices of rat cerebral cortex. Their results indicate that a high concentration (5×10^{-4} M) of pentobarbitone inhibited release stimulated by 25 mM K^+ to a greater extent than that due to 50 mM K^+.

In conclusion, evidence suggests that the effect of barbiturate anaesthetics on neurotransmitter release in-vitro depends on the stimulus used. However, both increases and decreases in release have been observed, depending on the neurotransmitter and the anaesthetic studied. Further studies on the mechanisms involved may reveal reasons for these discrepancies.

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Stimulation of brain dopamine autoreceptors by remoxipride administration in reserpine-treated male rats

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Abstract—Male Sprague-Dawley rats were treated subcutaneously with reserpine (5 mg kg^{-1}, −18 h) and with the aromatic amino acid decarboxylase inhibitor, NSD-1015 (3-hydroxybenzylhydrazine) (100 mg kg^{-1} −30 min). Remoxipride 0·8, 3·2 or 12·8 mg kg^{-1} was administered subcutaneously at −50 min. Immediately following decapitation (0 h), the ventral striatum and the anterior neocortex were dissected. Dopa and 5-hydroxytryptophan accumulation in these brain areas were analysed by HPLC with electrochemical detection. Reserpine produced a marked increase in striatal and neocortical dopa accumulation, in comparison with glucose vehicle + NSD-1015-treated controls, and this increase was dose-dependently antagonized by remoxipride treatment. Thus, together with demonstrated dopamine receptor antagonist actions in intact animals, remoxipride behaves as a mixed dopamine receptor agonist-antagonist. Such properties could contribute to the favourable endocrine and extrapyramidal side effect profile of remoxipride as an antipsychotic agent.

Remoxipride, [*S*(−)-3-bromo-*N*-[(1-ethyl-2-pyrrolidinyl) methyl] benzamide HCl, is a new dopamine D_2 receptor blocking agent (Florvall & Ögren 1982; Ögren et al 1984; Magnusson et al 1986) with antipsychotic properties (Chouinard 1987; McCreadie et al 1990). Well tolerated in healthy volunteers (Grind et al 1989), and with few or mild signs of extrapyramidal motor side-effects in clinical and laboratory studies (Ögren et al 1984), remoxipride shows potential as a new valuable antipsychotic agent (see Sedvall 1990).

The systemic administration of remoxipride produces increased neostriatal dopamine synthesis and turnover in the rat. Furthermore, remoxipride antagonizes dopamine-receptor-agonist-induced suppression of neostriatal dopamine synthesis in the γ-butyrolactone (GBL) model, a model where the dopamine synthesis is increased due to cessation of impulse flow and subsequent lifting of an autoreceptor-mediated tonic inhibition of dopamine synthesis and release (see Roth 1984). In both these preparations, however, the maximal effect of remoxipride appears less than that produced by haloperidol (Magnusson et al 1986, 1987, 1988). A less than maximal response is an effect expected by a partial dopamine agonist. Should this indeed be the case for remoxipride, the above observations, as well as the low propensity of remoxipride to produce extrapyramidal motor effects, could be explained by weak dopamine receptor stimulation intrinsic to the remoxipride treatment. In the present experiments, this possibility was investigated by the administration of remoxipride to rats pretreated with reserpine. The antagonism of reserpine-induced increase in brain dopamine synthesis, presumably mediated via presynaptic dopaminergic autoreceptors, has proven a sensitive index of dopamine agonist properties of dopaminergic agents (see Carlsson 1975; Roth 1984). Brain dopamine synthesis was therefore estimated by measuring the accumulation of dopa, following 3-hydroxybenzylhydrazine (NSD-1015), in reserpine-treated rats (Carlsson et al 1972).

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Materials and methods

Animals. Adult male Sprague-Dawley rats, 280–320 g, were supplied by ALAB Laboratorietjänst AB (Sollentuna, Sweden), and maintained under controlled conditions of temperature (20–21°C), relative humidity (55–65%) and a 12:12 h light-dark cycle (lights off 0600 h). The animals were acclimatized for at least 10 days before being used in experiments.

Drugs. The following drugs were used: reserpine (Fluka, Buchs, Switzerland), 3-hydroxybenzylhydrazine 2HCl (NSD-1015) (Sigma, St Louis, MO), haloperidol (Janssen, Beerse, Belgium), (−)-sulpiride (ICFI, Milan, Italy) and remoxipride HCl (batch F14) (Astra, Södertälje, Sweden). Reserpine, haloperidol and sulpiride were dissolved in a minimal quantitiy of glacial acetic acid, and the final volume was made up with 5·5% glucose. The other compounds were dissolved in physiological saline. All compounds were injected subcutaneously in a volume of 2 mL kg^{-1}. Controls received the corresponding amount of the vehicle. The doses refer to the drug forms indicated above.