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Stimulation by noradrenaline of inositol phospholipid breakdown in the rat hippocampus: effect of the ambient potassium concentration

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The stimulation by noradrenaline (NA) of inositol phospholipid (PI) breakdown has been studied using rat hippocampal miniprisms. Pretreatment with the monoamine oxidase inhibitor, pargyline, potentiated the stimulation produced by NA. On the other hand, pargyline pretreatment did not affect the stimulation of PI breakdown by the α_1-adrenoceptor agonist phenylephrine. NA- and phenylephrine-stimulated PI breakdown were enhanced by increasing the ambient potassium concentration in the assay from 5·88 to 18·2 mM. This enhancement did not, in the case of NA, change either the EC50 value for this agonist (2–3 μM) or the pA_2 value for the competitive antagonism of the stimulation by the α_1-antagonist prazosin (pA_2 value 9·2). Time-courses of the NA-stimulated PI turnover in different brain regions indicated that the rate of stimulation was in the order frontal cortex > hypothalamus ≥ hippocampus ≫ cerebellum.

Some recent studies have demonstrated that in the rat brain, several neurotransmitters and their agonists are able to stimulate the breakdown of inositol phospholipids to form inositol-1,4,5-triphosphate and diacylglycerol, both of which have been suggested to act as second messengers (for reviews, see Downes 1983; Berridge 1984). One such transmitter is noradrenaline (NA), which has been demonstrated to stimulate inositol phospholipid hydrolysis ('PI breakdown') in rat cerebral cortical slices via activation of α_1-adrenoceptors (Brown et al 1984; Minneman & Johnson 1984; Schoepp et al 1984). Little is known, however, about the effects of NA on PI breakdown in regions of the rat brain other than in the cerebral cortex, although a study by Janowsky et al (1984) demonstrated that NA-stimulated PI breakdown in rat hippocampal slices could be prevented by 100 nM prazosin. In addition, none of the above studies used monoamine oxidase inhibitor-pretreated tissue in order to prevent the breakdown of the exogenously applied NA by monoamine oxidase, raising the possibility that the potency of NA as a stimulator of PI breakdown in the rat brain has been underestimated in the literature.

In all the above studies, the assays were undertaken using a single ambient potassium concentration, usually about 5 mM. Increasing the potassium concentration at assay has been shown not only to affect the basal rate of PI breakdown (Brossard & Quastel 1963), but also to enhance greatly the stimulation of PI breakdown produced by the muscarinic agonist carbachol in cerebral cortical slices (Candy et al 1985). It was therefore considered necessary to determine whether this enhancement was specific to muscarinic receptor-coupled PI breakdown, or whether it could also be found for α_1-adrenoceptor-coupled PI breakdown. Thus, in the present study, the effect of the monoamine oxidase inhibitor pargyline and the effect of the ambient potassium concentration upon NA-stimulated PI breakdown has been determined both in cortical and non-cortical brain regions. A preliminary report of some of the present results has been published (Fowler et al 1985).

MATERIALS AND METHODS

Preparation of miniprisms

Male Sprague-Dawley rats (Anticimex AB, Sollentuna, Sweden), 150–250 g, were used. For the study using cerebral cortical miniprisms (Table 1), one animal per experiment was used. For the studies using hippocampal, frontal cortical, hypothalamic and cerebellar miniprisms, tissue from 4–12 animals were pooled. For all studies with cerebral cortex, hippocampus and hypothalamus, naive animals were used, whereas for most of the studies with frontal

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cortex and cerebellum, the animals were control groups for in-vivo experiments undertaken separately from the present study, and had received one saline injection 24 h before death.

The animals were decapitated, and the hippocampus, frontal cortex, hypothalamus and cerebellum regions dissected on ice as described by Jonsson et al (1982). In initial studies, the samples were kept on ice until the miniprisms were made, but it was found that better rates of incorporation of the [^{3}H]myo-inositol into the lipid and inositol phosphate fractions occurred when the regions were placed in ice-cold Krebs-Henseleit buffer solution until the miniprisms were made. Both methods, however, gave the same EC50 value for NA and pA_2 value for prazosin.

Miniprisms (350 × 350 μm) were sliced by use of a McIlwain tissue chopper, and then dispersed and washed several times with Krebs-Henseleit bicarbonate (KHB) buffer (NaCl 118·5, KCl 4·75, $MgSO_4$ 1·18, $CaCl_2$ 2·52, KH_2PO_4 1·18, $NaHCO_3$ 25, glucose 11·1 mM).

Assay of PI breakdown

PI breakdown was assayed by slightly modified methods of Berridge et al (1982) and Watson & Downes (1983). The miniprisms were incubated with myo-[2-^{3}H]inositol (10 μCi ml^{-1} miniprism suspension), in a volume of 2–5 ml depending upon the size of the experiment, under an atmosphere of 95% O_2: 5% CO_2, for 60 min at 37 °C. The samples were washed three times with warm KHB buffer, regassed with 95% O_2: 5% CO_2, reincubated for 10 min at 37 °C, and further washed three times. The samples were then regassed and reincubated for 10 min at 37 °C either in the absence or presence of 50 μM pargyline. The miniprisms were then washed three times with warm KHB.

Aliquots (30 μl, apart from the prazosin experiments, where 25 μl aliquots were used) of the miniprisms were pipetted into 15 ml assay tubes containing 6 μl of the agonist solution and 264 μl of KHB buffer containing 10 mM Li^+ and either 6 or 20 mM K^+, to give assay concentrations of 8·8 mM Li^+ and either 5·88 or 18·2 mM K^+, respectively. Isotonicity was preserved by concomitant reduction in the Na^+ concentration. The KHB also contained 0·1 mg ml^{-1} ascorbic acid, to prevent autooxidation of the NA. The tubes were gassed with 95% O_2: 5% CO_2 and incubated, unless otherwise stated, for 30 min at 37 °C. In all cases, the reactions were started by the addition of the miniprisms, so that there were no large temperature changes for the miniprisms after the incorporation phase. The reaction was stopped by the addition of 0·94 ml chloroform–methanol (1 : 2 v/v) and the tubes vortex mixed for 5 min using a SMI Model 2601 multi-tube vortexer (Scientific Manufacturing Industries, Emeryville, CA, USA). The phases were separated by the addition of 0·31 ml distilled water and 0·31 ml chloroform followed by 10 s vortex and centrifugation for 5 min at 3000 rev min^{-1} in a Wifug bench centrifuge. Aliquots (750 and 300 μl, respectively) of the aqueous and organic phases were taken. The organic phase aliquots, containing the labelled inositol phospholipids ('Lipid') were placed in scintillation vials and allowed to evaporate to dryness. The aqueous phase aliquots were placed in 15 ml plastic centrifuge tubes to which 0·5 ml Dowex-1 (X8, formate form) +1·5 ml distilled water were added. The samples were vortexed for 2 × 1min and 800 μl of the aqueous layer (containing [^{3}H]inositol) were taken, where appropriate, and placed in scintillation vials. The Dowex samples were washed three times with 2 ml distilled water, and the ^{3}H-labelled inositol phosphates ('InsP') eluted by the addition of 1 ml 0·1 M formic acid + 1 M ammonium formate followed by vortex for 5 min. Aliquots (800 μl) of the eluate were placed in scintillation vials. 10 ml Aquasol (New England Nuclear) was added to the vials, and the samples counted for radioactivity by liquid scintillation spectroscopy with quench correction. The d min^{-1} given in the results refer to the counts in the total fraction, not just in the aliquot.

Monoamine oxidase activities in untreated and pargyline-treated miniprisms were determined radiochemically as described elsewhere (Ask et al 1983).

Materials

[2-^{3}H]Myo-inositol (specific acitivity 10–20 Ci mmol^{-1}, in a sterile water solution) was obtained from New England Nuclear GmbH, Dreieich, West Germany. (−)-[8-^{14}C] Noradrenaline hydrogen tartrate was obtained from Amersham International plc., Amersham, UK. Prazosin hydrochloride was obtained from Pfizer, Brussels, Belgium. (−)-Noradrenaline bitartrate, Dowex-1 (X8, chloride form) and pargyline hydrochloride were obtained from Sigma Chemical Co., St. Louis, Mo., USA. All other reagents were standard laboratory reagents of analytical grade wherever possible.

RESULTS

Expression of units

PI breakdown has been estimated by measuring the accumulation of [^{3}H]inositol phosphates (InsP) after inhibition of inositol-1-phosphatase by lithium (Ber-

ridge et al 1982). Although most authors express their results as InsP d min^{-1}, the present data are generally expressed as InsP/(Lipid + InsP) since this ratio is independent of either the miniprism aliquot volume used or the incubation time used during the incorporation phase (Court et al unpublished results).

The effect of pargyline treatment on NA-stimulated PI breakdown in rat hippocampal miniprisms with and without pargyline (50 μM) pretreatment is shown in Fig. 1. The monoamine oxidase activities towards 50 μM NA were 14 and 0·54 pmol (30 μl aliquot)$^{-1}$ min^{-1} (means, n = 4) for the untreated and pargyline-treated miniprisms, respectively. A significant potentiation of the NA-stimulated PI breakdown was found at low NA concentrations for the pargyline-treated miniprisms (Fig. 1). This potentiation was also found using 1 μM NA and cortical miniprisms, whereas the stimulation of PI breakdown produced by the α_1-adrenoceptor agonist phenylephrine (1, 10 & 100 μM) was not affected by pargyline pretreatment (Table 1). In all the remaining experiments with NA, pargyline-pretreated miniprisms were used.

Enhancement of NA-stimulated PI breakdown in rat hippocampus by K^+

From the data shown in Fig. 1, it is apparent that at a [K^+] of 18·2 mM, both basal and particularly NA-stimulated PI breakdown are greater than at [K^+] = 5·88 mM. For both potassium concentrations, the EC50 for NA was in the range 2–3 μM, and maximal stimulation found at concentrations above 100 μM (Fig. 1). Combined data from several experiments with 100–125 μM NA are shown in Fig. 2. The same pattern of stimulation was found when the data were expressed as InsP d min^{-1} (although in general with higher relative standard deviations) (Fig. 2A) as when they were expressed as InsP/(Lipid + InsP) (Fig. 2C). A slight decrease in the lipid counts was found under conditions of maximum stimulation by NA at [K^+] = 18·2 mM (Fig. 2B), presumably because the rate of inositol phospholipid breakdown exceeded its rate of synthesis from inositol. When the values shown in Fig. 2 were expressed as % of basal PI breakdown (to compensate for the small increase in basal PI breakdown produced by the high [K^+]), the stimulation of PI breakdown by 100–125 μM NA was greater at 18·2 mM than at 5·88 mM [K^+] (247% vs 206%) (Fig. 2). Thus, increasing the assay potassium concentration not only increases the d min^{-1} recovered in the InsP fraction, but also

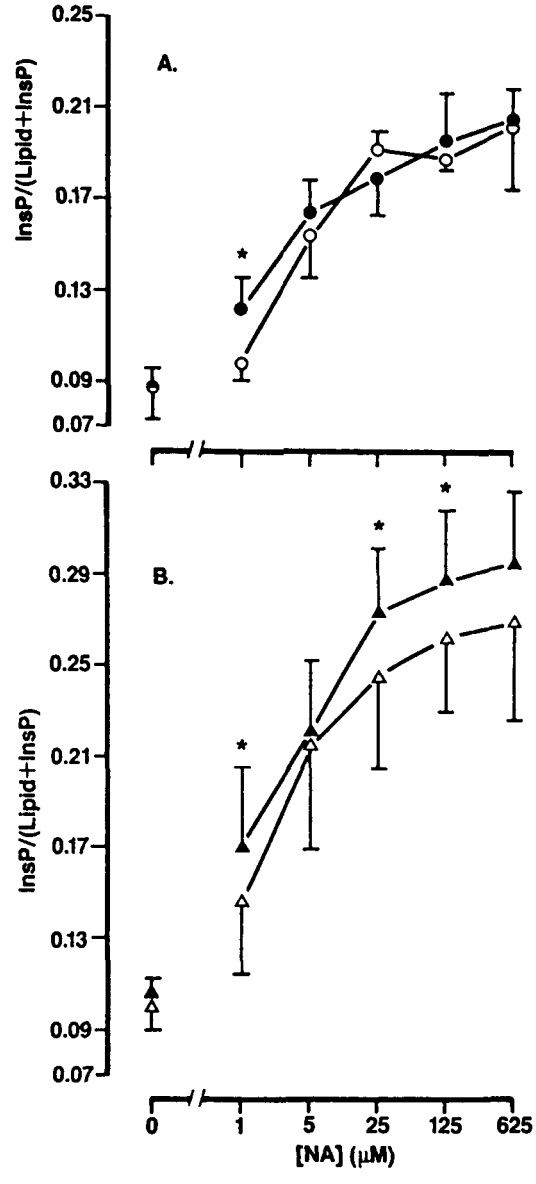

FIG. 1. NA-stimulated PI breakdown in rat hippocampal miniprisms with (●, ▲) or without (○, △) pretreatment with 50 μM pargyline. Data are means ± s.d. (n = 4). Samples were incubated for 30 min with NA at ambient potassium concentrations of either 5·88 mM (Panel A) or 18·2 mM (Panel B). *, Significantly different from the corresponding sample not treated with pargyline ($P < 0·05$, two-tailed paired *t*-test).

marginally enhances the stimulation produced by NA (Fig. 2) without altering the EC50 for NA (Fig. 1). This enhancement was also found for 1 μM NA and for phenylephrine (10 & 100 μM) in cerebral cortical miniprisms, but was in both cases much

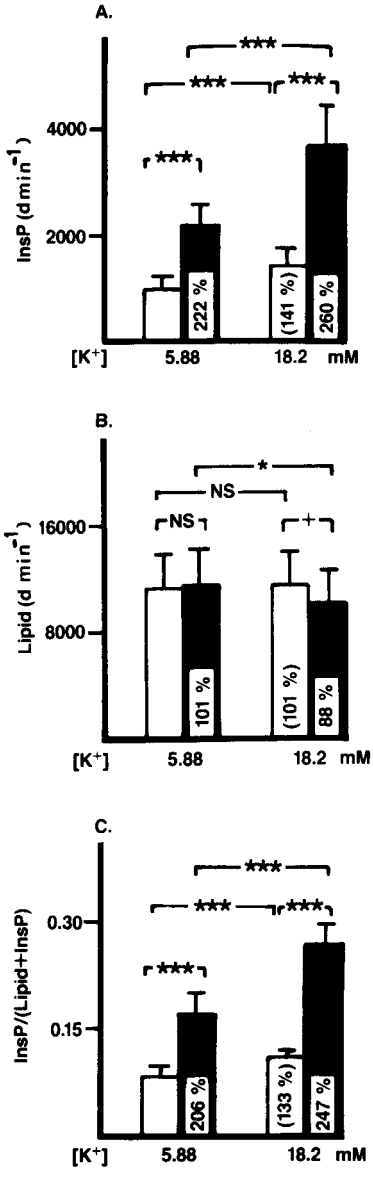

FIG. 2. NA-stimulated PI breakdown in rat hippocampal miniprisms. Samples were incubated for 30 min with ambient $[K^+]$ of either 5·88 or 18·2 mM, as indicated, either in the absence (unfilled columns) or presence of 100–125 μM NA (filled columns). Data are means ± s.d. (n = 10). The values enclosed within the filled columns are the mean stimulated values as a % of the mean basal value for that potassium concentration. For data as both InsP d min^{-1} and as InsP/(Lipid + InsP), the % stimulation was significantly greater at $[K^+]$ = 18·2 than at 5·88 mM ($P < 0·02$, Wilcoxon matched-pairs signed ranks test). The values in brackets in the unfilled columns are the mean basal values at $[K^+]$ = 18·2 mM as a % of the mean basal rate at $[K^+]$ = 5·88 mM. NS, not significantly different; +, $P = 0·062$; *, $P < 0·05$; **, $P < 0·01$ ***, $P < 0·001$, two-tailed paired *t*-test.

smaller than that found using the muscarinic agonist carbachol (100 μM) (Table 1).

Antagonism of NA-stimulated PI breakdown by prazosin

Preincubation of the prelabelled miniprisms for 30 min at 37 °C with the α_1-adrenoceptor antagonist prazosin was without effect on basal PI breakdown, but reduced in a dose-dependent manner the degree of stimulation of PI breakdown produced by NA. From the dose-response curves shown in Fig. 3, it can be concluded that the antagonism produced by prazosin is competitive in nature. Analysis of the curves by the method of Arunlakshana & Schild (1959) gave pA_2 values for prazosin of 9·15 and 9·17 for assay potassium concentrations of 5·88 and 18·2 mM, respectively (Fig. 3). Similar results were obtained when the data were calculated as InsP d min^{-1}, with pA_2 values calculated from the mean data of 9·15 and 9·38 for the assay $[K^+]$ of 5·88 and 18·2 mM, respectively.

NA-stimulated PI breakdown in different brain regions

Time courses of the stimulation of PI breakdown by 250 μM NA in miniprisms from hippocampus, frontal cortex, hypothalamus and cerebellum are shown in Fig. 4. From these data, it can be concluded that at 20 min incubation times, reasonable estimates of the rates of stimulation of PI breakdown by 250 μM NA at $[K^+]$ = 18·2 mM can be obtained for frontal cortex, hypothalamus and cerebellum, and up to 40 min (for both 5·88 and 18·2 mM $[K^+]$) for hippocampus.

Values for InsP d min^{-1}, inositol, Lipid and InsP/(Lipid + InsP) for the four regions after an incubation time of 20 min at $[K^+]$ = 18·2 mM are given in Table 2. Incorporation of the [^{3}H]myo-inositol into the lipid phase appeared to be greater for the frontal cortical, hypothalamic and cerebellar slices than for the hippocampal slices. The degree of stimulation (as % of basal values) of PI breakdown by 250 μM NA was similar for the data as InsP d min^{-1} as for InsP/(Lipid + InsP), and was in the order frontal cortex > hypothalamus ≥ hippocampus ≫ cerebellum. The rate of increase over basal of InsP/(Lipid + InsP) with time produced by 250 μM NA, calculated from these data (and in the case of the hippocampus also from the original data for Fig. 3) were: hippocampus (n = 7) 0·0053 ± 0·0007 min^{-1}; frontal cortex (n = 4) 0·0081 ± 0·0004 min^{-1}; hypothalamus (n = 4) 0·0058 ± 0·0003 min^{-1} and cerebellum (n = 5) 0·0006 ± 0·0004 min^{-1} (means ± s.d.).

Table 1. The effect of pargyline pretreatment upon the stimulation of PI breakdown by noradrenaline, phenylephrine and carbachol in rat cerebral cortical miniprisms.

		$[K^+]$ = 5·88 mM			$[K^+]$ = 18·2 mM		
[Pargyline] (μM)		0		50	0		50
Basal InsP/(Lipid + InsP)		0·051 ± 0·0090	–NS–	0·053 ± 0·0063	0·071 ± 0·011*	–*–	0·076 ± 0·011**
% of basal values in presence of:							
Noradrenaline	1 μM	123 + 4	–△–	150 ± 26	169 ± 11△	–△–	184 ± 17△
Phenylephrine	1 μM	117 ± 8	–NS–	120 ± 5	122 ± 12NS	–NS–	129 ± 19NS
	10 μM	153 ± 17	–NS–	158 ± 26	191 ± 24△	–NS–	195 ± 21△
	100 μM	185 ± 28	–NS–	172 ± 21	241 ± 23△	–NS–	225 ± 23△
Carbachol	100 μM	169 ± 21	–NS–	157 ± 8	422 ± 54△	–△–	383 ± 60△

Data are means ± s.d., n = 4. Significant differences between either the 50 μM pargyline and 0 μM pargyline-pretreated samples or alternatively between the $[K^+]$ = 5·88 mM and $[K^+]$ = 18·2 mM assay values are indicated in the table. *, $P < 0·05$; **, $P < 0·01$; NS, not significant; two-tailed paired *t*-test. △, 2P = 0·068 (max significance at n = 4); NS, not significant; Wilcoxon matched-pairs signed ranks test.

Table 2. Inositol phospholipid breakdown in miniprisms from rat hippocampus, frontal cortex, hypothalamus and cerebellum.

	NA	Brain region			
Parameter	(μM)	Hippocampus	Frontal cortex	Hypothalamus	Cerebellum
InsP (d min^{-1})	0	1250 ± 310	1990 ± 140	2470 ± 730	1990 ± 430
	250	2390 ± 180** (191%)	5530 ± 990** (278%)	5220 ± 870** (211%)	2250 ± 420** (113%)
Lipid (d min^{-1})	0	8300 ± 840	18740 ± 2120	17170 ± 4640	19670 ± 6580
	250	7630 ± 1140* (92%)	15790 ± 2700* (84%)	16580 ± 5020NS (97%)	19350 ± 6030NS (98%)
Inositol (d min^{-1})	0	39620 ± 4890	69540 ± 7010	118690 ± 39450	44940 ± 6680
	250	41060 ± 6520NS (104%)	65840 ± 6560NS (95%)	107730 ± 25830NS (91%)	45250 ± 6460NS (101%)
InsP/(Lipid + InsP)	0	0·133 ± 0·041	0·097 ± 0·010	0·130 ± 0·034	0·098 ± 0·026
	250	0·241 ± 0·031** (181%)	0·260 ± 0·010** (268%)	0·246 ± 0·032** (189%)	0·110 ± 0·028* (112%)

Data are means ± s.d. (n = 4–5). The incubation time with noradrenaline was 20 min, at an assay potassium concentration of 18·2 mM. Values in brackets are the mean values in the presence of 250 μM NA as a % of the mean values in the absence of NA. NS, not significant; *, $P < 0·05$; **, $P < 0·01$, two-tailed paired *t*-test with respect to the values in the absence of NA.

DISCUSSION

The present results indicate that in the rat hippocampus, NA stimulates PI breakdown, and that the breakdown can be potently antagonized by prazosin. The pA_2 for prazosin was about 9·2, which is in line with the K_d value for the binding of [^{3}H]prazosin to α_1-adrenoceptors, which is in the range of 0·06–0·4 nM (see e.g. Greengrass & Bremner 1979; Miach et al 1980; Menkes et al 1983; Clarkson et al 1984). Thus the data shown in Fig. 3 are in agreement with the conclusion drawn earlier for cerebral cortical miniprisms (Brown et al 1984; Minneman & Johnson 1984; Schoepp et al 1984) that NA stimulates PI breakdown in the rat brain via activation of α_1-adrenoceptors.

The rates of NA-stimulated PI breakdown from four regions were determined (Table 2, Fig. 4). The responses in frontal cortical, hippocampal and hypothalamic miniprisms were roughly similar, whereas only a very small degree of stimulation of PI breakdown by NA was found in cerebellar miniprisms. This small cerebellar response is rather suprising in view of the large NA concentration (Hallman & Jonsson 1984) and [^{3}H]WB 4101 (2-[*N*-(2,6-dimethoxyphenoxyethyl)]aminomethyl-1,4-benzodioxane) binding (U'Prichard et al 1977) found in cerebellar preparations. It can of course be argued that it is not relevant to compare binding data obtained in membrane preparations with PI breakdown data obtained in miniprisms. However, Minneman & Johnson (1984) have demonstrated a good correlation between the K_i values for the inhibition of NA-stimulated PI turnover in cerebral cortical miniprisms by α_1-adrenoceptor antagonists and their K_d values as displacers of the specific binding of [^{125}I]BE 2254 (2-[β-(4-hydroxyphenyl)ethylamino-

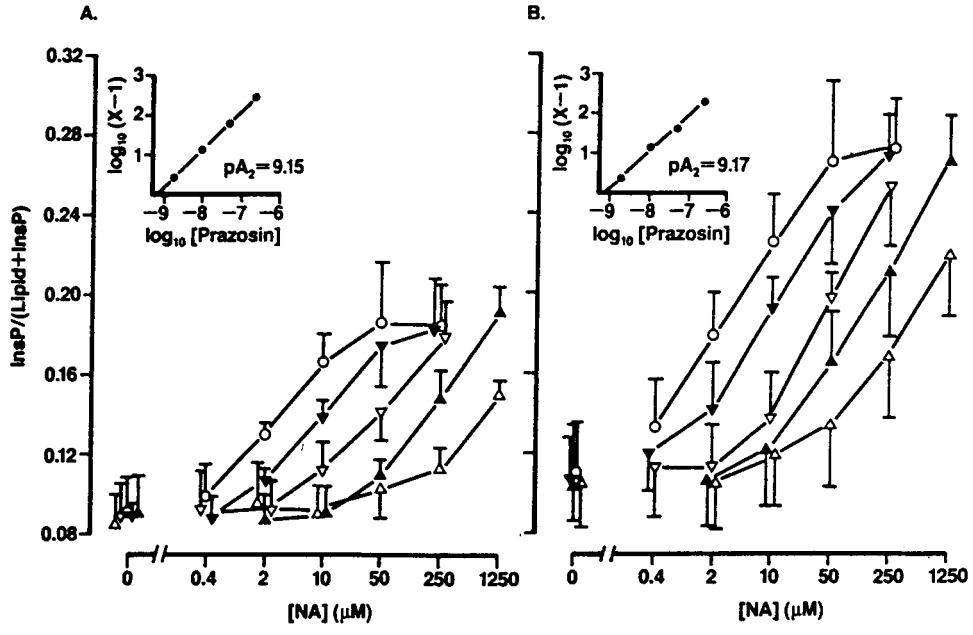

FIG. 3. Antagonism of NA-stimulated PI breakdown by prazosin. Samples were preincubated with either 0 (○), 2 (▼), 10 (▽), 50 (▲) or 250 nM (△) prazosin for 30 min at 37 °C in Krebs-Henseleit bicarbonate buffer containing Li^+ and either 5·88 mM (Panel A) or 18·2 mM (Panel B) $[K^+]$ before addition of NA and further incubation for 30 min at 37 °C. Data are means ± s.d., n = 3. Schild replots of the mean values are given as insets.

methyl]tetralone) to adrenoceptor sites in rat cortical membranes. Furthermore, the α_1-adrenoceptor occupancy of NA (determined from the NA displacement of [^{125}I]BE 2254 binding) was superimposable over the NA-stimulated PI turnover when both were expressed as % of maximum response (Minneman & Johnson 1984).

An alternative explanation for the difference between cerebellar NA-stimulated PI breakdown and cerebellar [^{3}H]WB 4101 binding is that the difference is simply a result of comparing an agonist-mediated response with antagonist binding (for discussion, see Menkes et al 1983), although in an analagous situation a good correlation was found between the rate of histamine-stimulated PI breakdown and the degree of the antagonist [^{3}H]mepyramine binding to histamine H_1-receptors (Daum et al 1983). It is unlikely that the small cerebellar PI response to NA is due to a loss of metabolic activity brought about by the procedures used in the prepara-

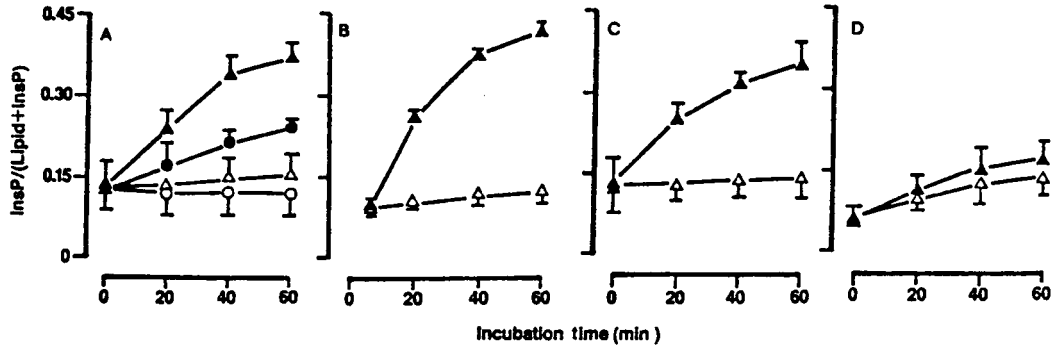

FIG. 4. Time courses of NA-stimulated PI breakdown in miniprisms from: A, hippocampus; B, frontal cortex; C, hypothalamus and D, cerebellum of the rat. Data are means ± s.d. (n = 4–5). Incubation conditions: $[K^+]$ = 5·88 mM in the absence (○) and presence (●) of 250 μM NA (Panel A alone); $[K^+]$ = 18·2 mM in the absence (△) and presence (▲) of 250 μM NA (Panels A–D).

tion of the miniprisms (see Marcusson et al 1985, for discussion) since such a loss of function would also affect the incorporation of the [^{3}H]inositol into the phospholipid phase, which does not seem to be the case (Table 2). It seems most likely that the binding of [^{3}H]WB 4101 to cerebellar membranes of the rat reflects binding to recognition sites not coupled to the PI response. In line with this conclusion is the recent finding that 3 nM[^{3}H]WB 4101 is able to label 5-HT S_1 binding sites in the rat brain (Norman et al 1985). Thus, NA-stimulated PI breakdown can be considered to be a more useful indicator of α_1-adrenoceptor function in-vitro than [^{3}H]WB 4101 binding measurements.

One important difference between the present study and previous investigations into NA-stimulated PI breakdown in the brain is that in the present study pargyline has been used to prevent metabolism of the exogenously applied NA by monoamine oxidase, and that ascorbic acid has been added to the Li^+-KHB buffer to prevent NA autooxidation. Whilst this is not strictly necessary at saturating NA concentrations, low concentrations of NA will be affected (Fig. 1, Table 1). This may be the explanation as to why in the present study a significant stimulation of PI breakdown was found with 1 μM NA whereas no such stimulation was found by other authors (Brown et al 1984; Minneman & Johnson 1984; Schoepp et al 1984) who did not use pargyline-pretreated slices. It would thus seem advisable to use miniprisms that have been pretreated (followed by extensive washing to remove the free inhibitor) with pargyline, and to include ascorbic acid in the assay medium when studying the stimulation of PI breakdown by low concentrations of NA. The use of pargyline may however be contraindicated when maximum responses to a number of neurotransmitter agonists (other than NA) are being tested in view of the slight attenuation of the carbachol-stimulated PI breakdown in the pargyline-pretreated cortical miniprisms at a $[K^+]$ of 18·2 mM (Table 1).

It has long been known that high (100 mM) concentrations of potassium result in an increased PI breakdown (Brossard & Quastel 1963). Recent studies, furthermore, have indicated that an increased potassium concentration considerably enhances the stimulation of PI breakdown in both cerebral cortex and hippocampal miniprisms produced by the muscarinic agonist carbachol, with an optimal enhancement being found at $[K^+] \simeq 20$ mM (Candy et al unpublished results). The present study indicates that NA-stimulation of PI breakdown is also enhanced by increasing the ambient potassium concentration from 5·88 to 18·2 mM without changing either the EC50 value for NA (2–3 μM) or the pA_2 value for antagonism by prazosin (Figs 1–3). This enhancement appears to be receptor- rather than agonist-related, since phenylephrine-stimulated PI break-down is enhanced by increased $[K^+]$ to the same extent as the NA-stimulated PI breakdown (Table 1).

It can be speculated that the enhancement of the muscarinic PI response may represent a mechanism amplifying the cellular response to a cholinergic stimulus under transient depolarizing conditions (when the extracellular $[K^+]$ is raised) and thus provide a mechanism for improving the signal: noise ratio of neurotransmission. The present study would furthermore suggest that such a mechanism is not universal for all neurotransmitter systems, since the enhancement of the α-adrenoceptor-mediated PI response by increased $[K^+]$ is much smaller than that found for the muscarinic receptor-mediated PI response (Table 1). However, the enhancement of NA-stimulated PI breakdown by increased $[K^+]$, albeit of marginal physiological importance, may be of use on a purely methodological basis, since it has the effect of increasing the observed d min^{-1} in the InsP fraction (particularly upon NA stimulation) without changing the pharmacological properties of the response.

Acknowledgements

To minimize the number of animals used, the experiments in the present study were done in conjunction with other studies using other brain regions. The authors are thus indebted to Prof. Svante Ross, Dr Håkan Hall and their research groups for their collaboration and help. The authors would also like to thank Ingrid Fagervall for undertaking the monoamine oxidase assays. During these studies, Dr O'Carroll was supported in part by the Swedish Medical Research Council (Grant no. 12X-5664).

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