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EFFECTS OF CATECHOLAMINES ON DOPAMINE AND SEROTONIN SYNTHESIS IN RAT BRAIN STRIATAL SYNAPTOSOMES: THE ROLE OF PRESYNAPTIC RECEPTORS AND THE SYNAPTOSOMAL REUPTAKE MECHANISM.

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ABSTRACT

The regulation of dopamine and serotonin synthesis in rat brain striatal synaptosomes has been studied using HPLC methods. Noradrenaline was shown to markedly inhibit both the synthesis of dopamine and serotonin. The response of the synaptosomes to the concentrations of noradrenaline appeared to be biphasic, a very effective inhibition occurring at low concentrations (1-5 μm) and a relatively ineffective further inhibition occurring at high concentrations (up to 100μM). The inhibition of dopamine and serotonin synthesis by noradrenaline was also studied in the presence of phenoxybenzamine (alpha adrenergic receptor blocker) and imipramine (reuptake inhibitor). Phenoxybenzamine changed the pattern of inhibition of both dopamine and serotonin synthesis by noradrenaline by preventing the very effective inhibition previously seen at low (1-5 μM) noradrenaline concentrations. Imipramine, whilst showing marked inhibition of dopamine synthesis on its own, prevented any inhibition by noradrenaline. In the case of serotonin synthesis, however, imipramine alleviated some of the inhibition seen in the presence of noradrenaline alone. The results are discussed with respect to the role that presynaptic receptors and reuptake mechanisms play in the regulation of catecholamine and serotonin synthesis at the nerve ending.

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INTRODUCTION

It is now widely recognized that the concentration of catecholamine neurotransmitters at the synapse are in the short term regulated via a negative feed-back mechanism involving tyrosine hydroxylase "E.C. 1.14.16.2". This hypothesis, which was originally developed from studies on tyrosine hydroxylase in cell

free preparations from a number of tissues,[2-4] suggests that catecholamine feedback and inhibit tyrosine hydroxylase activity by competing with the reduced pterin cofactor of that enzyme.

This feed loop involving catecholamines and tyrosine hydroxylase has also been studied in more integrated systems such as the synaptosome.[5-9] However, since synaptosomes contain the natural pterin cofactor[5-10] and retain the ability to take up exogenous catecholamines and store them in vesicles[11-13] and possess receptors on the presynaptic portion of the synapses,[14] the response of the synaptosomal tyrosine hydroxylase is necessarily more complex than in the cell free system.

In this study taking advantage of: (a) the much greater sensitivity afforded by use of the HPLC technique using electrochemical detection for the assay of catecholamines, and (b) a new more sensitive assay for tyrosine hydroxylase based on this technique[15] we have attempted to delineate the roles played by the various processes involved in the regulation of catecholamine synthesis at the level of the synapse (synaptosome). Since catecholamines appear to have a similar effect on tryptophan hydroxylase "E.C.l. 14.16.4", the first and probably rate limiting enzyme of serotonin synthesis,[16-17] we have also studied and compared the effects of catecholamines on the rate of serotonin synthesis in rat brain striatal synaptosomal preparations. Using phenoxybenzamine, a blocker of alpha adrenergic receptors[18] and imipramine, an inhibitor of reuptake of both catecholamines and serotonin,[19] we have studied the way in which dopamine, noradrenaline and serotonin inhibit the synaptosomal tyrosine and tryptophan hydroxylase's activity and how the presynaptic receptors and reuptake mechanisms may modify these processes.

MATERIALS AND METHODS

Materials

Tyrosine-HCl,typtophan, imipramine, adrenaline noradrenaline bitartrate, dopamine-HCl, 3,4-dihydroxybenzylamine (DHBA) and serotonin were purchased from Sigma (London) Chemical Company, Poole, Dorset, U.K. Catecholamine and serotonin stock solutions consisted of 100 μg/ml (free base) in 0.1 M perchloric acid of each compound stored at 4° C and freshly prepared every month. N-Methyl-N-propargyl-benzylamine-HCl (pargyline) and 2-amino, 6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropterine ($DMPH_4$) were obtained from Aldrich Chemical Company, The Brickyard, New Road, Gillingham, Dorset, U.K. Sodium octylsulphate was purchased from Cambrian Chemicals (Croydon), U.K. Carbidopa was a kind gift of Dr.M.E. Jaffe, Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania, U.S.A. This compound in a concentration of 4 μg/ml in 0.1M perchloric acid was used as internal standard for the measurement of serotonin in the synaptosomes.

Phenoxybenzamine-HCL (dibenyline) was a kind gift of Smith, Kline and French Laboratories, Welwyn Garden City, Herts, U.K. All other reagents used of the highest purity commercially available and were purchased from one of the aforementioned suppliers.

Preparation of Synaptosomal Fraction

Male Wistar rats (200-250g) were killed by decapitation and the brain was dissected by the method of Glowinski and Iversen.[20] The synaptosomes were prepared from the striatum as described by Boarder and Fillenz.[10] The incubation medium contained final concs. 125 mM-NaCl, 5 mM-KCl, 1 mM-$CaCl_2$, 1 mM-$MgCl_2$, 10 mM-glucose, 1 mM-ascorbic acid, 15 mM-sodium phosphate buffer to give final pH of 6.1 (optimal pH for tyrosine hydroxylase activity)[6,7] for determination of dopamine synthesis and 7.4 (optimal pH for tryptophan hydroxylase activity)[29] for serotonin synthesis. The determination of the synaptosomal protein concentration was performed by the method of Lowry, et al,[31] after suspending the synaptosomes in a 2% (w/v) Na deoxycholate solution (final concn.) and using bovine albumin as a standard. The protein concentration for synaptosomal preparation was about 7-12 mg/ml.

Measurement of Dopamine and Serotonin Synthesis

This was carried out by the methods previously described[9] and involved the determination of the levels of dopamine and serotonin by HPLC before and after incubation of the synaptosomes in the presence of a monoamine oxidase inhibitor (pargyline).

HPLC of incubation extracts was carried out on a 15cm × 4.6mm (i.d.) Ultraspher-IP column and detection by a LC-4 amperometric controller with a TL-4 detector (Bioanalytic Systems) linked to a Hewlett-Packard integrator recorder. Separation of the catecholamines was carried out by a mobile phase consisting of 90% 0.1 M potassium dihydrogen orthophosphate, 0.1 mM K^+-EDTA, 0.3 mM sodium octyl sulphate and 10% HPLC grade methanol (pH 3.0). For serotonin separation the same mobile phase but with a lower (0.03 mM) sodium octyl sulphate concentration was used.

RESULTS

Dopamine Synthesis

Fig. 1 indicates the effects that a range of noradrenaline concentrations (1-100 μM) has on the rate of dopamine synthesis in rat brain striatal synaptosomes. Dopamine synthesis is extremely sensitive to noradre-

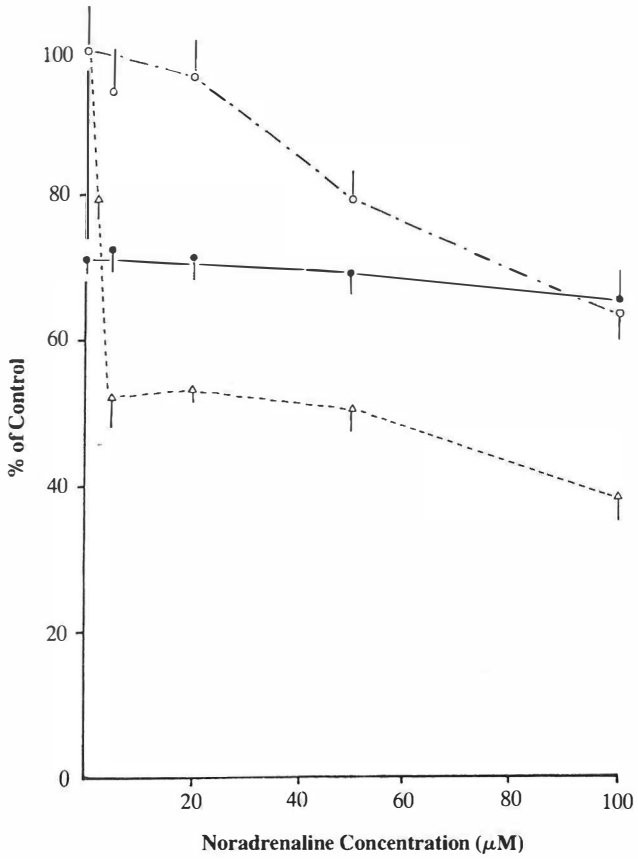

Fig. 1. Effect of phenoxybenzamine and imipramine on inhibition of dopamine synthesis by noradrenaline in rat brain synaptosomes.

Noradrenaline (△-----△) 1-100 μM was added to the striatal synaptosomal suspensions and incubated for 15 min. at 37°C in the presence of 40 μM tyrosine and 100 μM pargyline. In certain cases 200 μM phenoxybenzamine (○----○) or imipramine (●—— ●) were also added. The rate of dopamine synthesis was measured as described in the Methods. Each point represents a mean ± S.E.M. from 4-6 estimations expressed as a % of control.

Control values (100%) for dopamine synthesis were 14.1± 0.3 (n = 16) pmols. dopamine synthesised/min/mg. Synaptosomal protein (mean ± S.E.M.). Significant difference of noradrenaline concs. higher than 5 μM from control P< 0.005.

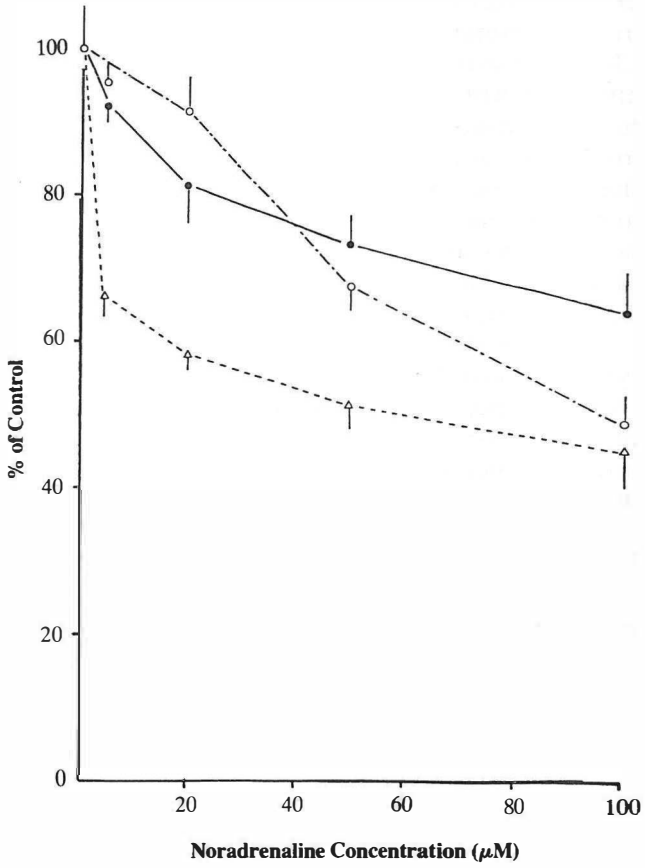

Fig. 2. Effect of reduced pterin cofactor on inhibition of dopamine synthesis by noradrenaline in striatal synaptosomes.

Noradrenaline alone (△ --- △) or noradrenaline + 50 μM DMPH$_4$ (2 amino-4 hydroxy-6, 7-dimethyl-5,6,7,8 tetra hydropterin) (▲——▲) were added at the concentration indicated (0-100 μM) to the striatal synaptosomal preparation and incubated with 40 μM tyrosine in the presence of 100 μM pargyline for 15 min. at 37°C. Dopamine synthesis was measured by HPLC as described in the Methods section. Each point represents a mean ± S.E.M. of 3-6 points expressed as a % of the control.

Control values (100%) for dopamine synthesis were 13.2 ± 0.4 (n = 12) pmols/min/mg synaptosomal protein (mean ± S.E.M.).Significant difference of noradrenaline concs. higher than 5 μM from control P <0.005.

naline in the range of 1-5 μM, being 50% inhibited at approximately 5 μM concentration. Further increase in noradrenaline concentration up to 100 μM only causes a further 10% inhibition. It is clear from Fig. 1 that the effects of noradrenaline on dopamine synthesis are biphasic, there being a very sensitive region of inhibition in the 1-5 μM range followed by a much less effective region up to 100 μM. This raises the possibility that more than one inhibitory mechanism is involved in controlling the rate of dopamine synthesis. This was further investigated by studying in more detail the effects of noradrenaline on dopamine synthesis in the presence of phenoxybenzamine, a blocker of alpha adrenergic receptors,[18] and imipramine, a blocker of high affinity uptake system of catecholamines across the nerve plasma membrane.[19] The presence of 200 μM phenoxybenzamine clearly change the pattern of inhibition of dopamine synthesis by noradrenaline. In the presence of phenoxybenzamine concentrations of noradrenaline up to 20 μM had no significant effect on dopamine synthesis and at 100 μM noradrenaline it was only inhibited by 30% (values of dopamine synthesis in the presence of phenoxybenzamine and noradrenaline as compared with those in the presence of noradrenaline alone; at noradrenaline concentrations of 5 and 20 μM, P< 0.005, at concentrations of 50 and 100 μM, P< 0.05.). In short there is a loss of the very sensitive region of inhibition observed in the presence of noradrenaline alone. In the presence of imipramine, howev-

er, the change of pattern of inhibition is quite different, in that dopamine synthesis is significantly inhibited (30%) even in the absence of any added noradrenaline and addition of noradrenaline up to 100 μM causes no further inhibition i.e. there is a loss of the less effective inhibition seen at high noradrenaline concentrations in the presence of noradrenaline alone (values of dopamine synthesis in the presence of imipramine and noradrenaline as compared with those in the presence of noradrenaline alone, at all noradrenaline concentrations, $P < 0.05$).

Since the inhibitory effect of catecholamines on tyrosine hydroxylase has been reported to be competetive with respect to reduced pterin cofactor[3,4] the effect of $DMPH_4$ (a synthetic analogue of the cofactor) on the inhibitory effect of noradrenaline on the rate of dopamine synthesis by the synaptosomes was examined (Fig.2). In the absence of noradrenaline, 50μM of $DMPH_4$ was found to increase dopamine synthesis by about 20% ($P<0.05$). However, when noradrenaline in the range of concentration from 5 to 100 μM was added to the synaptosomes in the presence of $DMPH_4$ (50 μM) the pattern of inhibition was not significantly different to that in its absence.

Serotonin Synthesis

The effect of noradrenaline on serotonin synthesis in striatal synaptosomes as well as the modifying effects of phenoxybenzamineand imipramine on the noradrenaline induced inhibition of serotonin synthesis is shown in Fig.3. Noradrenaline showed a similar pattern of inhibition of serotonin systhesis (see Fig. 1) exhibiting a very marked inhibition at low noradrenaline concentrations (35% inhibition at 5 μM noradrenaline $P<0.05$ with only a marginally greater inhibition at higher concentrations (50% inhibition at 100 μM noradrenaline, $P<0.005$). Again the pattern of inhibition by noradrenaline of serotonin synthesis in the presence of phenoxybenzamine (200 μM) was significantly different to that in its absence ($P<0.005$ for noradrenaline concentrations = 5 μM: $P<0.05$ for concentrations 20 and 50 μM). The concentration relationship was similar to that seen on dopamine synthesis (see Fig.1 and 3) in that there was a loss of the very effective inhibition of serotonin synthesis at low noradrenaline concentrations (5 μM) but was different in that at 100 μM noradrenaline concentration the degree of inhibition of serotonin synthesis was approximately the same whether phenoxybenzamine was present or not. Imipramine however appeared to decrease the effectiveness of noradrenaline induced inhibition of serotonin synthesis over the whole concentration range, showing relatively little inhibition at 5 μM noradrenaline (showing only 6-7% inhibition in the presence of imipramine as compared to 35% in its absence) but at 100 μM noradrenaline 35% inhibition plus imipramine and 55% inhibition in the absence of imipramine (Fig.3).

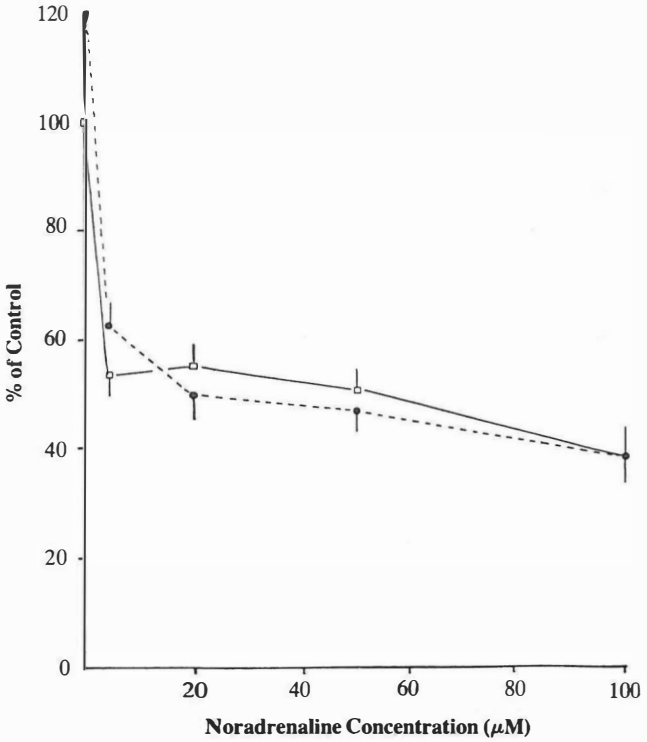

Fig. 3. Effect of phenoxybenzamine and imipramine on the inhibition of serotonin synthesis by noradrenaline in rat brain synaptosomes.
Noradrenaline (△ --- △) 1-100 μM was added to the striatal synaptosomal suspensions and incubated for 40 min. at 37°C in the presence of 60 μM tryptophan and 100 μM pargyline. In certain cases 200 μM phenoxybenzamine (○...○) or imipramine (●—●) were also added. The rate of serotoninsyntheis was measured by HPLC as described in the methods. Each point represents a mean ± S.E.M. from 4-6 points expressed as a % of control.
The control value (100%) for serotonin synthesis was 2.30 ± 0.07 (n = 18) pmols serotonin synthesis/min/mg synaptosomal protein (mean ± S.E.M.). Significant difference of noradrenaline concs. higher than 20 μM from control $P<0.005$.

DISCUSSION

The response of the striatal synaptosomal tyrosine and tryptophan hydroxylases to inhibition by noradrenaline had a distinct biphasic concentration relationship. There was a very marked and significant decrease in the rate of dopamine and serotonin formation observed at low noradrenaline concentrations (< 5 μM) which was not observed when phenoxybenzamine was present. At higher noradrenaline concentrations (5 μM) there was a much less effective inhibition of dopamine/serotonin synthesis which was still seen in the presence of phenoxybenzamine. This suggests that 2 mechanisms may be involved in the noradrenaline

induced inhibition of dopamine and serotonin synthesis, a) a mechanism involving a presnyaptic receptor which is extremely sensitive to the noradrenaline concentration and which is blocked by the presence of phenoxybenzamine, b) a much less sensitive mechanism which may involve a more direct effect of noradrenaline on the tyrosine and tryptophan hydroxylases. The existence of presynaptic receptors capable of modulating synaptic function have been proposed both for peripheral[18,21,22] and central nervous systems.[23-24] In vivo studies by Walters and Roth[25] have also suggested that there are presynaptic receptors on dopaminergic nerve terminals which may modulate the sensitivity of tyrosine hydroxylase to end product inhibition. The mechanism by which this occurs is still controversial but may be related to the binding of tyrosine hydroxylase to the synaptic memebrane and the modulation of its activities by this association.[1] In particular it has been reported that the membrane bound tyrosine hydroxylase has a higher affinity for the pterin cofactor and tyrosine and that Ca^{2+} may influence the association of the enzyme with the membrane.[26-28]

Another finding in these experiments is the effect of $DMPH_4$ on the inhibitory activity of noradrenaline on dopamine synthesis in synaptosomes. As shown in Fig. 2 the presence of $DMPH_4$ caused little effect on the response of the synaptosomes to noradrenaline. The possibility that the cofactor is not entering the synaptosome is unlikely since in the absence of inhibitor (noradrenaline) dopamine synthesis is increased by about 20% when $DMPH_4$ alone is added. This suggests that the effects that are being observed here (see Figs. 1-3) are not directly related to cofactor binding properties of the tyrosine hydroxylase.

A further complication in respect of the complexity of the synaptosomal response to the inhibitory action of noradrenaline on tyrosine and tryptophan hydroxylases lies in the ability of the synaptosomes to take up catecholamines[11,12] and store them in synaptic vesicles.[13] These uptake mechanisms are inhibited by imipramine and similar compounds[19] which as a consequence will decrease the concentration of endogenous noradrenaline in the synaptosol in these experiments. This may explain the general decrease in the effectiveness of noradrenaline in its inhibition of serotonin synthesis (see Fig. 2). It does not however explain the 30% or more inhibition of dopamine synthesis by imipramine observed in the absence of any added noradrenaline (Fig. 1). This suggests an alternative effect of imipramine which gives rise to increased accumulation of endogenous dopamine/noradrenaline which in turn feed back and inhibit tyrosine hydroxylase. Imipramine may also by a similar mechanism give rise to increased levels of free endogenous serotonin. However, it is unlikely that this will effect serotonin synthesis very markedly as serotonin is a relatively poor inhibitor of tryptophan hydroxylase[29-30] in contrast to dopamine which is a potent inhibitor of tyrosine hydroxylase activity.[7]

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