Effect of antidepressants on extracellular catecholamine concentrations in the rat hippocampus using in vivo microdialysis

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Abstract:
The effect of antidepressants on extracellular norepinephrine (NE) and dopamine (DA) levels were measured in hippocampus of freely moving rats using the microdialysis technique combined to a radioenzymatic assay. The NE basal level was of $4.6 \pm 0.2$ pg/30 µl of dialysat; the DA basal level was in the limit of detection i.e. about 1 pg/30 µl. Acute single intraperitoneal administration of various antidepressants produced an increase of NE basal level after 30 min which was observed during 1 to 3 h. These changes in NE levels were quantitatively different according to the antidepressant used with the following potency rank order: tranylcypromine (32 mg/kg) > nomifensine (16 mg/kg) > viloxazine (32 mg/kg) > mianserin (16 mg/kg) > amitriptyline (32 mg/kg) > desipramine (32 mg/kg). Citalopram did not produce any increase in NE level. Only nomifensine and tranylcypromine could increase DA levels to a high extent. These results confirm previous data obtained in vitro on brain slices and on synaptosomes preparations and bring new informations about the magnitude and the duration of the variations of catecholamine levels as observed in vivo.

Key-words: Antidepressants, dopamine, hippocampus, microdialysis, norepinephrine, radioenzymatic assay.

The assessment of modifications of neurotransmission in CNS is greatly facilitated by measurements of neurotransmitter extracellular concentrations in vivo. The technique of in vivo microdialysis (Delgado et al., 1972; Ungerstedt, 1991) offers several advantages over the traditional “push-pull” cannula technique. Dialysis probes allow the measurement of neurotransmitter concentrations in discrete brain areas of small animals (Hernandez et al., 1986) and the combination of microdialysis with highly sensitive analytical techniques allows the measurement of a lot of neuroactive compounds in vivo (Benveniste, 1989; Di Chiara, 1990). Antidepressant drugs inhibit either NE, DA and 5-HT neuronal uptake or the monoamine oxdase enzymes (MAO), leading to an increase of the amine concentrations in the synaptic cleft (Richelson, 1984; Potter et al., 1991). In attempt to compare the effects of different antidepressants on cerebral catecholamines in vivo, we have evaluated changes in NE and DA extracellular concentrations induced by one administration of antidepressants in the hippocampus, using the brain microdialysis technique in freely moving rats.
Materials and methods

In vivo microdialysis

Male Wistar rats (200-300 g) were anaesthetized with chloral hydrate (400 mg/kg), i.p.) and mounted in a stereotaxic apparatus (David Kopf). The dialysis probe (outer diameter 0.50 mm ; MW cut off 20000 ; 14 mm in total length ; dialysis tip of 4 mm ; CMA/12, (Carnegie Medicin, Phymep, Paris, France) was implanted into the hippocampus according to the following coordinates with respect to bregma: anterior, 5.8 mm; lateral, 5.0 mm; ventral, 3.2 mm (stereotaxic atlas, Paxinos and Watson, 1986). The probe was secured to the skull with screws and dental cement (Superbond) and the overlying skin was sutured. Experiments began the following day after implantation. The dialysis probe was connected to a microinfusion pump (Carnegie Medicin, CMA/100) and continuously perfused with modified Ringer’s solution (147 mM NaCl, 4 mM KCl, 2.3 mM CaCl2, pH 6.5) at a flow rate of 1.5 µl/min. Twenty-minutes fractions (30 µl) of the dialysate were collected into 400 µl polyethylene microtubes (Eppendorf) containing 3 µl of 0.25 M EGTA and 0.20 M reduced glutathione, adjusted at pH = 7 with 1N NaOH, and then immediately frozen and kept at -80°C until analysis. The position of the microdialysis probe was verified on 250 µm thick frozen coronal sections of the brain.

All experiments began at 9 a.m. in order to avoid nycthemeral variations. After introduction of the probe, no sample was collected during one hour in order to stabilize the catecholamine levels (Kalen et al., 1988). Then after, 5 samples were collected in order to determine the basal level of catecholamines and then drugs were intraperitoneally administered and samples were collected for 4 to 5 hours in 20-min fractions.

This protocol meets the guidelines of the French agency regarding animal experimentation (authorization n°00748 delivered to Pr. J-P. TILLEMENT).

In vitro recovery experiments

Recovery of NE and DA was determined by in vitro experiments, at room temperature. Dialysis probes placed in vials containing 10^-7 M each of NE and DA dissolved in Ringer’s solution/ antioxidant (10/1, v/v) were perfused with Ringer’s solution (see above) at a constant rate flow of 1.5 µl/min. After dialysate levels were stabilized (approximately one hour), three twenty minutes fractions were collected and analyzed for catecholamine content.

Recovery was computed as the ratio of the catecholamine concentration found in the dialysate to that present in the test solution. The catecholamine mean relative recoveries of the dialysis probes were found to be 24.5 ± 2.2 % (mean ± S.E.M. of 3 probes). Biochemical assays NE and DA concentrations were determined in the dialysates using a commercial radioenzymatic assay kit (CAT-A-KIT Assay, Amersham, France) sensitivity: 1 to 2 pg) according to the method of Peuler and Johnson (1977).

Statistical analysis:

Data are expressed as mean ± S.E.M. of the percentage increase or decrease over baseline levels. Baseline values were calculated as the mean percentage of the 5 first sequential samples for each animal. At least 5 animals were used for controls and treatments. Statistical significance between controls and treated groups was assessed using a paired Student’s t-test, with p<0.05 being considered as statistically significant.

Reagents and drugs:

EGTA, dopamine HCl, norepinephrine HCl and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA).

The following drugs were kindly provided by the companies indicated and administered
intraperitoneally (2 ml/kg) : amitriptyline HCl (Roche), desipramine HCl (Ciba-Geigy), tranylcypromine (RBI), viloxazine HCl (ICI), citalopram HBr (Lundbeck), mianserin HCl (Organon) and nomifensin maleate (Hoechst). NaCl 9 g/l (2 ml/kg) was administered intraperitoneally to a control group of five rats. All other chemicals were of analytical grade and were obtained from Merck (Darmstadt, Germany).

Results

• Basal release of norepinephrine and dopamine.
  The basal level of NE measured in the dialysate from the hippocampus was 4.6 ± 0.2 pg/30 µl (n = 37 ; 25 % recovery). When control rats received saline solution (9 g/l NaCl, 2 ml/kg, i.p.), the NE concentration in the dialysates remained stable over a 3h-period and was close to the basal level, indicating that the stress induced by the injection did not affect the NE basal release.
  The basal level of DA measured in the dialysate was 20- to 30-fold lesser than that of NE (Bischoff et al., 1979) and could not be easily detected ; however the DA basal level could be measured in some rats (n=5) and was evaluated to 1 – 4 pg/30 µl (25 % recovery).
• Effect of antidepressants on noradrenaline release
  Desipramine (32 mg/kg) produced a 3.3-fold increase in extracellular levels of NE. The maximal effect of the drug was observed after 40 min, maintained for 20 min and then decreased progressively (Fig. 1). After 300 min, a 2-fold increase in NE level was still observed. A similar pattern was observed with amitriptyline (32 mg/kg), viloxazine (32 mg/kg) and mianserin (16 mg/kg) which increased extracellular levels of NE by 3.8-, 3.95- and 3.35- from the basal level, respectively (Fig.1). Nomifensine (16 mg/kg) was the most active antidepressant, with a maximal peak (8.2-fold) delayed and observed after 140 min, without recovering to the basal level after 300 min (5-fold) (Fig.1). Tranylcypromine (32 mg/kg) induced a rapid and large increase in NE level up to 28-fold with a rapid basal level recovery after 140 min (Fig.1) whereas citalopram (5 mg/kg), a specific inhibitor of the serotonin uptake, did not induce any significant change in NE level (data not shown).
• Effect of antidepressants on dopamine release.
  Desipramine, amitriptyline, viloxazine and mianserin did not modify the DA basal level. In contrast, nomifensine caused a 4-fold increase in DA basal level 20 min after its administration ; a plateau was observed between 120 and 240 min and the basal level did not recover after 300 min (Fig. 2). A similar pattern was observed with tranylcypromine (32 mg/kg) which induced a 26-fold increase in DA level 120 min after its administration (Fig. 2).
• Effect of antidepressants on behavioral modifications
  The implantation of the probe was followed by a short locomotor activity, then a gradual sedation set up. Saline solution (0.9 % NaCl), amitriptyline, desipramine, viloxazine and citalopram did not induce any behavioral modification. Mianserin induced a strong sedation and nomifensine an increase in the locomotor activity with stereotypies such as head swingings, snifflings and nibblings. These behavioral modifications appeared 40 min after the administration of the drug and persisted during 150 min and vanished at the end of the microdialysis. Tranylcypromine caused a delayed locomotor activity (1 h after administration) showing convulsions, respiratory failure and hyperthermia, sometimes leading to the death of the animal. A good correlation was set up between
Fig. 1: Effect of different antidepressants on extracellular NE levels in the rat hippocampus. The i.p. administration of 0.9 % NaCl (○) or drugs is indicated by the arrow. The perfusion rate into the dialysis probe was 1.5 µl/min. NE was measured in 20-min fractions. The mean basal level of NE was 4.60 ± 0.20 pg/20 µl. Each point represents the mean ± S.E.M. of values obtained from 5 rats.

DESI: desipramine (32 mg/kg); VILO: viloxazine (32 mg/kg); AMI: amitriptyline (32 mg/kg); MIAN: mianserin (16 mg/kg); NOMI: nomifensin (16 mg/kg); TRAN: tranylcypromine (32 mg/kg).

*p<0.05; **p<0.01 versus the mean basal level of NE.
behavioral modifications and changes in NE and DA levels with mianserin and tranylcypromine.

**Discussion**

These data show that in vivo microdialysis coupled to a high sensitive radioenzymatic assay allows to measure NE and DA extracellular levels in the rat hippocampus after acute administration of antidepressants. Noradrenergic and dopaminergic innervations constitute the main monoamine pathways in the hippocampus. In this structure, the DA level is about 1000-fold lower than that observed in the striatum and 40 % of DA found in the hippocampus would be contiguous to noradrenergic neurons (Bischoff et al., 1979). Because the hippocampus plays an important role in the affective disorders, it constitutes a privileged area to study the mechanism of action of antidepressants. It was shown that chronically administered antidepressants down-regulate beta-adrenoceptors in the cerebral cortex and the hippocampus but not in the caudate nucleus, the olfactory tubercles or the substantia nigra (Anwyl and Rowan, 1984; Biegon, 1986). In our study, all antidepressants tested increased significantly the NE level in the hippocampus, except citalopram which is known to possess a high selectivity for the serotonergic system (Hyttel, 1982).

Desipramine is a very potent and specific blocker of NE uptake (Sugrue, 1981; Richelson and Pfenning, 1984). Previous experiments performed on rat brain synaptosomes have shown that the administration of 32 mg/kg desipramine inhibits 80 % of the NE uptake (Manias and Taylor, 1983). We observed that desipramine administered at the same dose increased by 4-fold the basal level of NE. This effect probably resulted from the inhibition of NE uptake. It was previously shown, by means of transcortical dialysis in

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**Fig. 2. Effect of different antidepressants on the extracellular DA levels in the rat hippocampus.**

The i.p. administration of 0.9 % NaCl (c) or drugs is indicated by the arrow. The perfusion rate into the dialysis probe was 1.5 µl/min. DA measured in 20-min fractions. The mean basal level of DA was 1.16 ± 0.22 pg/30 µl. Each point represents the mean ± S.E.M. of values obtained from 5 rats.

NOMI : nomifensin (16 mg/kg) ;
TRAN : tranylcypromine (32 mg/kg).

*p<0.05 ; ** p<0.01 versus the mean basal level of DA.
but, as an antagonist of presynaptic alpha2-adrenoceptors (Baumann and Maitre, 1977), it increases the NE turn-over in the rat brain (Kafoe et al., 1979). A comparable release of NE was observed in vivo in the rat brain cortex after administration of idazoxan, a selective alpha2-adrenoceptor antagonist (L’Heureux et al., 1986; Dennis et al., 1987). Thus, it is likely that the mianserin-induced release of NE observed in our experiments results from an inhibition of alpha2-adrenoceptors.

Nomifensine inhibits potently NE and DA uptakes although it shows a tenfold lower affinity for the DA carrier (Morin et al., 1989). However, nomifensine is twofold more efficient than desipramine and amitriptyline to stimulate the release of NE in the hippocampus. This suggests that in addition to the blockade of NE uptake, nomifensine could act through another mechanism which could be relevant to its amphetamine-like psychostimulant properties (Schacht and Heptner, 1974). In return, the effect of nomifensine on the release of DA in the hippocampus (Fig. 2) corresponds entirely to its potent inhibitor effect on DA uptake (Hunt et al., 1974).

Tranylcypromine is a racemate where the d-isomer inhibits more potently MAO B than MAO A, this latter deaminating preferentially NE. However, at the dose of 32 mg/kg, tranylcypromine inhibits both enzymes as well as the NE and DA uptakes (Tuomisto and Smith, 1986). These combined mechanisms could explain the strong variations of monoamine levels observed in our experiments. We show that the efflux of NE or DA takes place progressively after the administration of tranylcypromine, as previously reported by Finberg (1987). A similar delayed effect was also reported with pargyline, another MAO inhibitor (Imperato and Di Chiara, 1984; Sharp et al., 1986).

In conclusion, these data demonstrate that i.p.
administration of tricyclic, atypical or MAO inhibitor antidepressant drugs differently affect extracellular NE and DA levels in the rat hippocampus and are consistent with the regulation of extracellular NE and DA content in the hippocampus by neuronal uptake and to a lesser extent by presynaptic autoreceptors.

References

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