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FAILURE BY TRICYCLIC ANTIDEPRESSANTS TO AFFECT THE INCREASE OF DOPAMINE EXTRACELLULAR CONCENTRATIONS PRODUCED BY HALOPERIDOL IN THE CAUDATE AND ACCUMBENS NUCLEI OF RATS

PAOLA DEVOTO,* GRAZIELLA DE MONTIS and ALESSANDRO TAGLIAMONTE†
Institute of Pharmacology, University of Siena, Via delle Scotte, 6, 53100 Siena; and *Department of Neuroscience, University of Cagliari, Via Porcell, 4, 09124 Cagliari, Italy

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Abstract—The effect of different inhibitors of monoamine uptake on dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) neuronal outflow was studied in the caudate and accumbens nuclei of rats, using the *in vivo* brain microdialysis method coupled to HPLC electrochemical detection. Under conditions of DA receptor blockade (as produced by the i.p. administration of 0.25 mg/kg of haloperidol), cocaine, GBR-12909 and *d*-amphetamine increased the concentration of extracellular DA beyond the effect produced by haloperidol alone in both areas studied. GBR-12909 and cocaine also increased DOPAC concentration, while *d*-amphetamine decreased it. On the contrary, the tricyclic antidepressants (TCA), desipramine and chloripramine, failed to modify the effect of haloperidol on DA and DOPAC neuronal outflow. It was concluded that: (a) noradrenergic and serotonergic nerve terminals do not take up DA released from dopaminergic neurons, and (b) TCA have no effect on dopaminergic terminals.

Long-term exposure to imipramine (IMI‡) and repeated electroconvulsive shock produce in rats a down regulation of dopamine (DA) D_1 receptor number [1] and a decrease in adenylate cyclase response to DA stimulation [2], in the limbic areas. On the contrary, both treatments increase the V_{max} of adenylate cyclase in the same areas [3] measured by forskolin or guanylyl imiolooliphosphate activation. All these effects are prevented by the daily co-administration with IMI of a small dose of α-methyl-*p*-tyrosine [1, 3], which has no effect *per se* on the variable studied. It was concluded that an active synthesis of catecholamines is needed for the down regulation of the D_1 receptor–adenylate cyclase complex induced by chronic antidepressant treatments. Moreover, it was proposed that the changes observed are the consequence of an increased DA concentration in the synaptic cleft. This hypothesis is supported by the finding that IMI and, particularly, the metabolite which mediates its effect in rats, DMI [4, 5], inhibit the uptake of [^{3}H]DA in synaptosomes prepared from limbic areas (which are rich in both noradrenergic and dopaminergic terminals) with a potency 100-fold greater than that observed in the basal ganglia [1]. The IC_{50} for [^{3}H]DA uptake inhibition of DMI is within the concentration range observed in the brains of rats treated with antidepressants [6] at the dosage schedule used in the reported studies. DA uptake inhibition was used to explain the finding that in rats chronic IMI decreases selectively the DOPAC concentration in the limbic system [1]. In fact, DOPAC is the deaminated metabolite of DA which seems to reflect the amount of amine recaptured by nerve terminals [7]. However, this explanation does not agree with the fact that acute IMI administration does not modify limbic DOPAC concentration (unpublished results). Moreover, since DMI is a specific inhibitor of monoamine uptake at noradrenergic nerve endings [8], the question arises as to whether limbic noradrenergic nerve endings take up only the DA co-released with noradrenaline, or also that released by dopaminergic terminals.

Both questions were addressed by studying, with microdialysis technique, the effect of different monoamine uptake blockers on extracellular DA and DOPAC concentrations in the brain of animals pretreated with haloperidol. The neuroleptic was administered at a dose that maximally increases DA and DOPAC release from dopaminergic nerve terminals, in order to minimize the contribution of noradrenergic terminals to both DA and DOPAC concentration outside neurons.

Under such experimental conditions TCA failed to modify the extracellular concentrations of both DA and DOPAC, while cocaine and the other compounds known to inhibit monoamine uptake at dopaminergic terminals all increased DA concentration and showed distinct effects on DOPAC levels.

MATERIALS AND METHODS

Male Sprague–Dawley rats (Charles River, Italy), body weight 280–320 g, were anesthetized with chloralium hydrate (400 mg/kg, i.p.) and placed in a Kopf stereotaxic frame. The skull was exposed and a hole was drilled through the two temporal bones, for the insertion of the transversal dialysis

† Corresponding author. Tel. (39) 577-280896; FAX (39) 577-286202.

‡ Abbreviations: DA, dopamine; DOPAC, dihydroxyphenylacetic acid; IMI, imipramine; DMI, desipramine; TCA, tricyclic antidepressants; GBR-12909, [2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine.

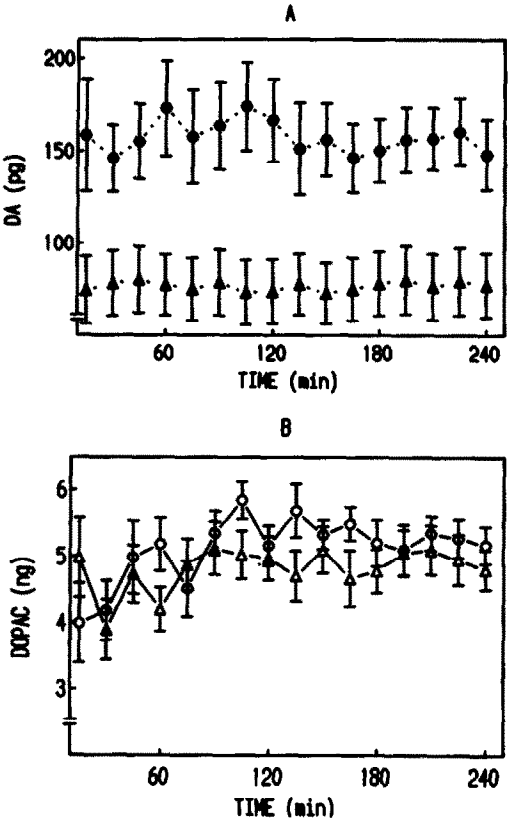

Fig. 1. Time-course of DA (A) and DOPAC (B) in perfusate collected from caudate (circles) and accumbens (triangles) of rat not treated with drugs. Each point is the mean ± SEM of data from five rats.

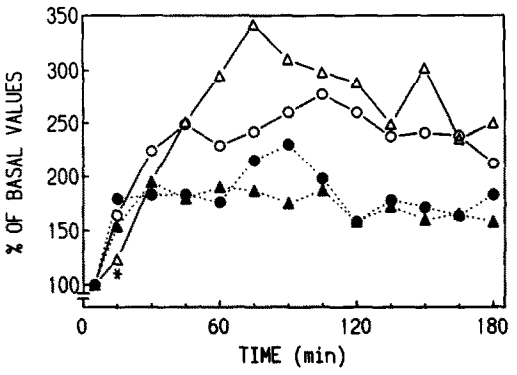

Fig. 2. Effect of haloperidol on DA (filled symbols, dotted lines) and DOPAC (open symbols, solid lines) release in perfusate collected from caudate (circles) and accumbens (triangles) of rats. Each point is the mean of data from four rats. The SEM do not exceed 10% of values. The 100% value indicates the mean of the 4–5 stable measurements before haloperidol injection. * $P > 0.05$. All other values were significantly different from basal release ($P < 0.05$).

probe. Coordinates, relative to bregma, were: A +1.0, V −5.0, for nucleus caudatum; A +1.2, V −7.0, for nucleus accumbens, according to the Paxinos and Watson [9] atlas. The dialysis probe was prepared and inserted according to Imperato and Di Chiara [10]. The dialysis fiber (310 μm o.d., 220 μm i.d.; Hospal, Italy) was covered with superepoxy glue except for a 1-cm part for the caudatum and a 0.5-cm part for the accumbens, corresponding to the dialysing tracts. Twenty-four hours after surgery, the rats were connected to a CMA/100 Microinjection Pump (Carnegie Medicine), delivering 2 μL/min of a Ringer solution (147 mM NaCl, 3.4 mM $CaCl_2$, 5 mM KCl, pH 6–6.5 with 0.05 M NaOH). The perfusate collected during the first 30 min was discarded, then 30-μL samples were collected every 15 min into Eppendorf minitubes; the same volume of 0.1 N HCl was added to prevent oxidation and the samples were frozen at −70° until processed.

DA and DOPAC were determined by injecting 40-μL samples with a Waters autoinjector (WISP 712) into a HPLC apparatus equipped with a reverse phase ODS column (C18, Beckman) and an electrochemical detector (Waters 460). Chromatograms were analysed by a Waters 745 Data Module integrator. The mobile phase was a 0.1 M sodium acetate buffer, pH 4.6, containing 50 mg/L EDTA, 15 mg/L sodium octyl sulphate and 2% methanol. DA and DOPAC retention times were 8.5 and 6 min, respectively. Under these conditions, the limit of sensitivity was approximately 5 pg.

Due to individual differences between animals, data are expressed as the percentage of mean basal value for each treatment, obtained from 4–5 samples collected prior to drug injection. Statistical analysis was performed with the Dunnett's test, from the computer program Pharmacological Calculation System, version 4 (based on Ref. 11).

RESULTS

Figure 1 shows DA and DOPAC baseline levels in freely moving rats, chronically implanted with a microdialysis cannula as described in Materials and Methods. Before treatment animals were allowed a 60-min equilibration period, during which basal levels of DA and DOPAC were determined in 15-min (30 μL) dialysed samples.

Figure 2 shows the effect of haloperidol (0.25 mg/kg, s.c.) on DA and DOPAC output, both in the caudate nucleus and the nucleus accumbens, during the first 3 hr after injection. As expected [12], extracellular DA increased markedly in both nuclei and the effect was maximal during the 2nd hr. Moreover, the percentage increase in DOPAC beyond the basal values was even more pronounced and followed the same time-course as that of DA.

Figure 3 shows that cocaine, injected i.p. 60 min after haloperidol at the dose of 10 mg/kg, almost doubled the increase in extracellular DA above the haloperidol effect in both nuclei. In addition cocaine, whose effect lasted about 1 hr, slightly though not significantly potentiated the haloperidol-induced increase in the interstitial DOPAC concentration.

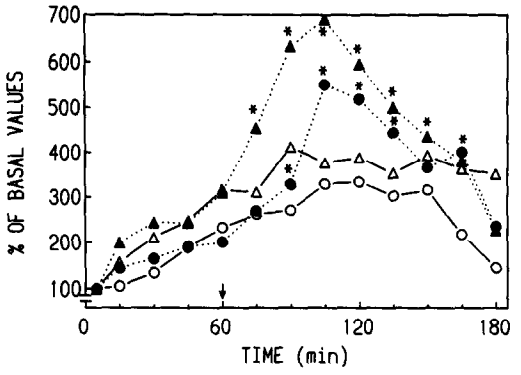

Fig. 3. Time-course changes induced by cocaine on haloperidol-stimulated DA (filled symbols, dotted lines) and DOPAC (open symbols, solid lines) release in perfusate collected from caudate (circles) and accumbens (triangles) of rats. Arrow indicates the time of injection of cocaine. Each point is the mean of data from four rats. The SEM do not exceed 14% of values. The 100% value indicates the mean of the 4–5 stable measurements before haloperidol injection. * $P < 0.05$ with respect to haloperidol values.

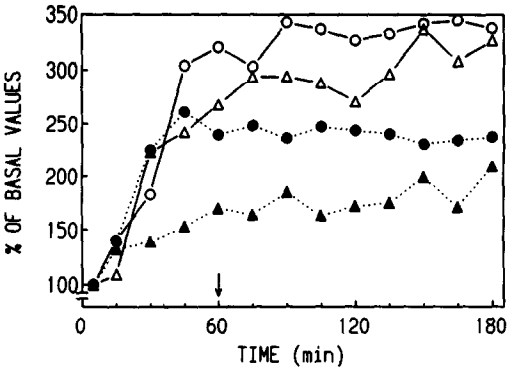

Fig. 5. Haloperidol-stimulated DA (filled symbols, dotted lines) and DOPAC (open symbols, solid lines) release in perfusate collected from caudate (circles) and accumbens (triangles) of rats after DMI administration. Arrow indicates the time of injection of DMI. Each point is the mean of data from four rats. The SEM do not exceed 10% of values. The 100% value indicates the mean of the 4–5 stable measurements before haloperidol injection. Curves are not significantly different from those of haloperidol.

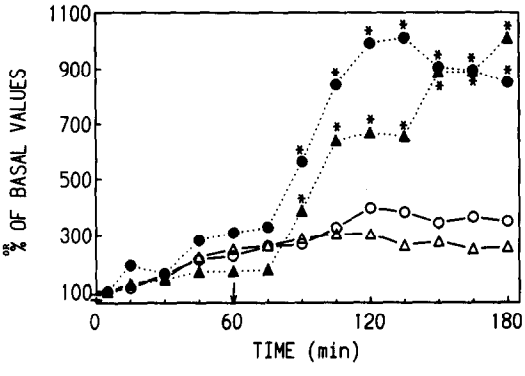

Fig. 4. Time-course changes induced by GBR-12909 on haloperidol-stimulated DA (filled symbols, dotted lines) and DOPAC (open symbols, solid lines) release in perfusate collected from caudate (circles) and accumbens (triangles) of rats. Arrow indicates the time of injection of GBR-12909. Each point is the mean of data from four rats. The SEM do not exceed 12% of values. The 100% value indicates the mean of the 4–5 stable measurements before haloperidol injection. * $P < 0.05$ in respect to haloperidol values.

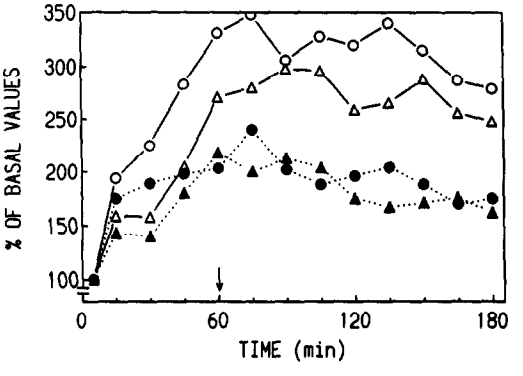

Fig. 6. Haloperidol-stimulated DA (filled symbols, dotted lines) and DOPAC (open symbols, solid lines) release in perfusate collected from caudate (circles) and accumbens (triangles) of rats after chlorimipramine administration. Arrow indicates the time of injection of chlorimipramine. Each point is the mean of data from four rats. The SEM do not exceed 10% of values. The 100% value indicates the mean of the 4–5 stable measurements before haloperidol injection. Curves are not significantly different from those of haloperidol.

As reported in Fig. 4, GBR-12909 injected i.p. at the dose of 10 mg/kg 60 min after haloperidol, produced effects analogous to those of cocaine, but of longer duration.

On the other hand, both DMI (Fig. 5) and chlorimipramine (Fig. 6) given at the dose of 10 mg/kg i.p. failed to significantly modify the time-course of DA and DOPAC output produced by haloperidol in both brain areas. Finally, Fig. 7 shows that *d*-amphetamine (4 mg/kg, i.p.) markedly increased extraneuronal DA concentration above the haloperidol effect both in the caudate and accumbens nuclei, while it decreased DOPAC concentration to basal values in the two areas.

DISCUSSION

Cocaine, *d*-amphetamine, and GBR-12909, all compounds known to inhibit DA reuptake at dopaminergic terminals, produced a marked increase in extracellular DA concentration beyond that produced by haloperidol, both in the caudate nucleus

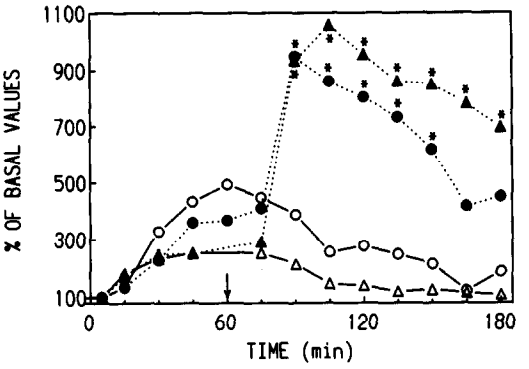

Fig. 7. Time-course changes induced by *d*-amphetamine on haloperidol-stimulated DA (filled symbols, dotted lines) and DOPAC (open symbols, solid lines) release in perfusate collected from caudate (circles) and accumbens (triangles) of rats. Arrow indicates the time of injection of *d*-amphetamine. Each point is the mean of data from four rats. The SEM do not exceed 15% of values. The 100% value indicates the mean of the 4–5 stable measurements before haloperidol injection. * $P < 0.05$ in respect to haloperidol values.

and in the accumbens. On the other hand, DMI and chlorimipramine, which rather selectively inhibit noradrenaline [8] and serotonin [13] uptake by the respective neuronal systems but have no effect on dopaminergic nerve endings, failed to modify the time-course of DA and DOPAC output produced by haloperidol.

These results do not support the hypothesis that noradrenergic or serotonergic nerve terminals, even in those brain areas where the respective neuronal systems are particularly well represented, play a relevant role in the uptake of the DA released from dopaminergic neurons.

Moreover, they exclude any effect of TCA on DA uptake by dopaminergic neurons.

Thus, the increased dopaminergic transmission observed in the limbic areas of rats chronically exposed to antidepressant treatments [1, 3] may find an explanation in: (a) a reuptake inhibition of the DA co-released with noradrenaline by noradrenergic nerve endings after TCA, (b) an increase in DA co-release parallel to that of noradrenaline, as observed after electroconvulsive shock [14] or monoamino oxidase inhibitors [15], and (c) a transynaptic activation of DA neurons due to an enhanced serotonergic and/or noradrenergic transmission.

The present data seem to exclude the hypothesis [1] that the decrease in DOPAC concentration in the limbic areas of rats chronically treated with IMI might be related to the uptake inhibition of DA released by dopaminergic neurons. In fact, IMI is a rather selective uptake blocker at the serotonergic terminals and DMI, the metabolite which is considered to mediate IMI effects in rats, specifically inhibits uptake at noradrenergic terminals; however, even with maximal DA output from dopaminergic neurons, as produced by haloperidol, the blockade of each of these uptake systems failed to affect the extracellular DA and DOPAC concentrations. Thus, at present we have no explanation for the decrease in limbic DOPAC after chronic IMI treatment.

It is of interest that both cocaine and GBR-12909 failed to decrease and rather had the tendency sometimes to increase DOPAC overflow beyond that caused by haloperidol, both in the caudate and in the accumbens. In fact, although cocaine alone increases homovanillic acid concentration [16], it has been reported to slightly decrease extracellular DOPAC in the accumbens. A possible mechanism for the increased extracellular DOPAC above the haloperidol effect caused by cocaine, and GBR-12909 is that the blockade of DA reuptake attenuates the end-product inhibition exerted by the monoamine on a tyrosine hydroxylase activity already stimulated by haloperidol [17]. This effect might have favored a further increase in DA synthesis and metabolism.

Finally, the expected [18] decrease in DOPAC concentration produced by *d*-amphetamine in both areas studied may be accounted for by the well known monoamine oxidase inhibitory activity of this compound [19].

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