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THE EFFECT OF DESIPRAMINE ON THE UPTAKE OF MESCALINE BY CORTICAL SYNAPTOSOMES

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Abstract—The uptake of ^{14}C-labelled noradrenaline and mescaline into a synaptosome-rich preparation from the rat cerebral cortex was studied. Noradrenaline was accumulated by the synaptosome-rich preparation by a temperature-dependent process which was competitively inhibited by desipramine. Mescaline was accumulated by a temperature- and sodium-dependent process. The uptake of mescaline was not affected by desipramine. The uptake of noradrenaline was inhibited by mescaline in a non-competitive manner; this suggests that the uptake of mescaline is not brought about by the noradrenaline uptake mechanism.

The uptake blockade hypothesis of potentiation proposes that the potentiation of responses to monoamines by tricyclic antidepressant drugs (e.g. desipramine) results from the ability of these drugs to block the accumulation of monoamines by presynaptic nerve terminals [1]. There are observations in the central nervous system which are consistent with this hypothesis. For example, it is known that desipramine can block the uptake of the monoamine noradrenaline (NA) into nerve terminals in the brain [2], and it has been shown that responses of single cortical neurones to NA can be potentiated by desipramine [3].

Using the technique of microelectrophoresis we have shown that the hallucinogenic monoamine mescaline (3, 4, 5-trimethoxyphenylethylamine) can evoke responses in cortical neurones which are similar to those evoked by NA [4]. Furthermore, responses of single cortical neurones to mescaline can be potentiated by desipramine [5]. It would be of interest to determine whether this potentiation of neuronal responses to mescaline by desipramine can be accounted for by the blockade of uptake into nerve terminals. In the experiments reported in this paper we have examined whether mescaline can be accumulated into synaptosomes prepared from the rat cerebral cortex. As a control the uptake of NA was also studied.

MATERIALS AND METHODS

Preparation of synaptosomes. Experiments were performed using male albino Wistar rats weighing 250–350 g. The rat was sacrificed by decapitation, the brain removed and the cerebral cortex dissected from the rest of the tissue on ice. The cerebral cortex was immediately homogenised in 0.32 M sucrose (10 ml) using a loose-fitting Teflon pestle and glass vessel. The blood and cellular debris were separated by centrifugation at 1000 *g* for 10 min at 4°. The supernatant was removed and centrifuged at 17,000 *g* for 5 min at 4°. The resultant synaptosome-rich pellet (see [6]) was suspended in cold mammalian Ringer solution (70 ml) [7].

Incubations. Synaptosome-rich Ringer solution (4.9 ml) was added to each incubation flask. In addition, "control" flasks contained a 0.9% w/v solution of NaCl (0.05 ml) while "test" flasks contained a solution of the test drug (0.05 ml). The synaptosomes were pre-incubated for 5 min, after which a solution of ^{14}C labelled monoamine (0.05 ml) was added to each flask. The monoamine added was either NA ((−)—noradrenaline—carbinol [^{14}C]bitartrate, sp. act. 5.0 m Ci/m-mole: Radiochemical Centre Ltd.) or mescaline (mescaline-8-[^{14}C]hydrochloride, sp. act. 5.2 m Ci/m-mole; New England Nuclear Corporation Inc.). Preliminary experiments showed that the rate of uptake of both mescaline and NA was constant over 20 min (see also [8].) Consequently, the incubation period used in these experiments was 10 min.

Incubations were performed at both 37° and 4°. The difference between the uptake at these two temperatures was considered to be the temperature-dependent (active) uptake of the monoamine.

Incubations were stopped by pouring the contents of each flask into chilled tubes followed immediately by centrifugation at 15,000 *g* for 5 min at 4°. The resulting pellet was washed by resuspending in 0.9% w/v NaCl (5 ml) and the centrifugation repeated. The supernatant was discarded and the pellet blotted dry.

Assay procedures. The washed pellet was homogenised in 0.4 N perchloric acid (1 ml) and the homogenate allowed to stand for 30 min at room temperature. The protein thus precepitated was removed by centrifugation at 15,000 *g* for 5 min at 4°. An aliquot of the supernatant (0.5 ml) was added to a glass scintillation vial, containing 1 N NaCl (0.2 ml) to neutralise the acid, and scintillation fluid added (10 ml). The scintillation fluid contained 0.267% 2,5-diphenoxyloxazole (PPO) and 0.0067% 1,2 bis-(5-phenoxyloxazole) benzene (POPOP) in toluene with 33% Triton X-100. Radioactivity was measured using a Packard liquid scintillation spectrometer where counting efficiency was monitored by the channel-ratio method.

The precipitated protein was dissolved in 2 N NaCl

(5 ml) by heating at 100° for 30 min. The protein content of this solution was estimated according to the method of Lowry, Rosebrough, Farr and Randall [9].

Graphical treatments. For the analysis of the relationship between uptake velocity and concentration of monoamine (NA or mescaline), rectangular hyperbolae were fitted to data using non-linear regression analysis [10]. An estimate of the goodness of fit of the curve to the data is given by the index of determination (p^2) (p^2 expresses the proportion of the variance of the y-values (ordinate) which can be accounted for in terms of the x-values (abscissa) in a curvilinear function [11].) Using Wilkinson's method [10], estimates for the maximum uptake velocity (V_m) and for the monoamine concentration giving rise to the half-maximum uptake velocity (K_m), along with the respective standard errors of these estimates, could be obtained. The estimated values of these parameters could be compared statistically using the normal t-distribution.

RESULTS

Uptake of noradrenaline. Figure 1(a) (open circles) shows, for different NA concentrations in the incubation medium, the temperature-dependent initial velocity of NA uptake into the synaptosome-rich preparation. The NA uptake velocity was an increasing negatively accelerating function of the NA concentration. The estimated values for V_m and K_m are shown in Table 1.

Uptake of mescaline. Figure 1(b) (open circles) shows, for different mescaline concentrations in the incubation medium, the temperature-dependent initial velocity of mescaline uptake. The mescaline uptake was an increasing negatively accelerating function of the mescaline concentration. The estimated values for V_m and K_m are shown in Table 1. The sodium dependency of mescaline uptake was investigated by incubating the synaptosome rich pellet in a sodium-free mammalian Ringer solution. Table 2 shows that the removal of sodium from the incubation medium resulted in a statistically significant reduction ($P < 0.001$, Student's t-test) in the uptake velocity of mescaline at both mescaline concentrations tested. Indeed, the uptake velocity of mescaline at 37° in sodium-free Ringer solution did not differ significantly ($P > 0.2$, Student's t-test) from the uptake of mescaline at 4° in normal (physiological) Ringer solution.

Effect of desipramine on the uptake of noradrenaline. In these experiments the concentration of desipramine in the incubation medium was 0.05 μM. Figure 1A (closed circles) shows that, in the presence of desipramine, the uptake of NA could still be described by a rectangular hyperbola. From this curve the apparent kinetic constants (V_m' and K_m') for the uptake of NA in the presence of desipramine could be calculated. Table 1 shows that desipramine did not affect the maximum velocity of NA uptake ($P > 0.2$); however, the K_m' was significantly different from the K_m

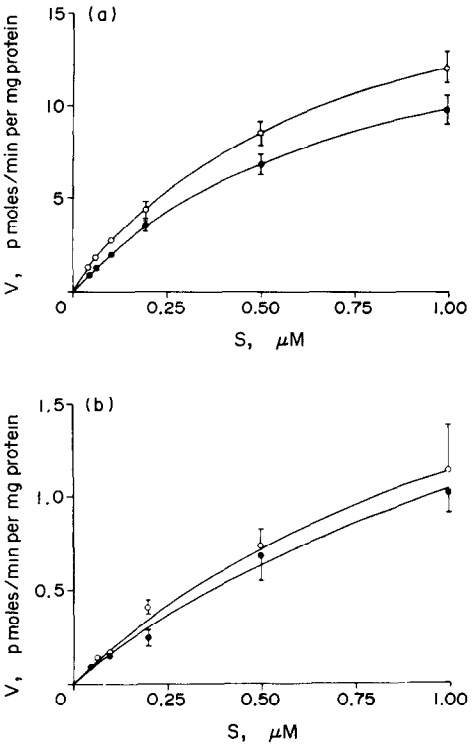

Fig. 1. Plot of the temperature-dependent uptake velocity (V) against the substrate concentration (S). Each point represents the mean of at least 4 determinations. Vertical bars indicate S.E.M. Curves were fitted by regression analysis [10].

(a) The effect of desipramine on the uptake of noradrenaline. Open circles: NA control; index of determination (see Methods) $p^2 = 0.999$. Closed circles: NA in the presence of 0.05 μM desipramine; $p^2 = 0.999$.

(b) The effect of desipramine on the uptake of mescaline. Open circles: mescaline control; $p^2 = 0.996$. Closed circles: mescaline in the presence of 0.05 μM desipramine; $p^2 = 0.994$.

Table 1. Kinetic constants for noradrenaline and mescaline uptake

Treatment		Noradrenaline	Mescaline
Control	K_m	0.63 ± 0.05	1.24 ± 0.27
	V_m	19.2 ± 0.7	2.63 ± 0.87
Desipramine	K_m'	0.83 ± 0.06*	1.43 ± 0.58
	V_m'	17.8 ± 0.8	2.88 ± 0.59
Mescaline	K_m''	0.48 ± 0.08	—
	V_m''	8.48 ± 0.7*	—

Estimates (±S.E.) for the kinetic constants K_m (μM) and V_m (pmoles/min/mg. protein) were obtained according to the method described by Wilkinson [10].

* $P < 0.001$. See text for details.

Table 2. The uptake of mescaline in sodium-free conditions

Mescaline concentration S (μM)	Mescaline uptake velocity V (pmoles/min/mg protein)		
	4° Normal Ringer	37° Na^+-free Ringer	37° Normal Ringer
0.500	0.65 ± 0.04	0.69 ± 0.03	1.29 ± 0.07
0.066	0.06 ± 0.01	0.08 ± 0.01	0.21 ± 0.02

Each value of V is the mean (± S.E.M.) of at least 4 determinations.

($P < 0.001$) for NA uptake. Thus, desipramine competitively inhibited the uptake of NA, and, using the analogy of enzyme kinetics [12], the K_i for desipramine was calculated to be 0.049 ± 0.003 μM.

Effect of desipramine on the uptake of mescaline. Figure 1B (closed circles) shows that, in the presence of 0.05 μM desipramine, the uptake of mescaline can be described by a rectangular hyperbola. From this curve the apparent kinetic constants for mescaline uptake in the presence of desipramine were calculated (see Table 1). Neither the K_m nor the V_m for the uptake of mescaline were significantly changed ($P > 0.2$) by the presence of 0.05 μM desipramine in the incubation medium.

Subsequently, the effect on the uptake of mescaline of increasing concentrations of desipramine was studied. The concentration of mescaline in the incubation medium was either 0.1 or 1.0 μM and the desipramine concentration was in the range 0.5–5.0 μM. Figure 2 is a plot of the reciprocal of the uptake velocity of mescaline against the desipramine concentration [13]. At both mescaline concentrations tested, the uptake velocity of mescaline was not affected by desipramine; linear regression analysis showed the two lines to be approximately parallel to the abscissa.

Thus, desipramine, within the concentration range 0.05–5.0 μM, did not inhibit the temperature-dependent uptake of mescaline.

Effect of mescaline on the uptake of noradrenaline. Figure 3 shows that, in the presence of 10 μM mescaline, the uptake of NA can be described by a rectangular hyperbola. From this curve, the apparent kinetic constants for the uptake of NA in the presence of mescaline (V_m'' and K_m'') were calculated. Table 1 shows that K_m'' did not differ significantly ($P > 0.05$) from K_m for NA uptake. However, mescaline decreased the maximum velocity of NA uptake, as V_m'' was significantly different from V_m ($P < 0.001$). This is consistent with the criteria for non-competitive inhibition, and, using the analogy of enzyme kinetics [12], the K_i for mescaline was calculated to be 10.55 ± 0.81 μM.

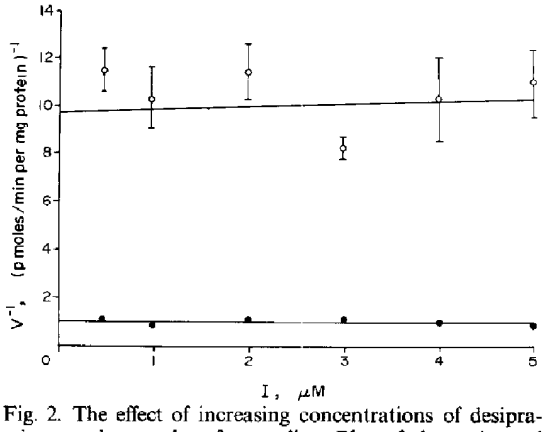

Fig. 2. The effect of increasing concentrations of desipramine on the uptake of mescaline. Plot of the reciprocal of the temperature-dependent uptake of mescaline (V^{-1}) against the concentration of desipramine (I) [13]. Each point is the mean of at least 4 determinations (as in previous figures). Open circles: mescaline (0.1 μM); correlation coefficient, $r = 0.279$. Closed circles: mescaline (1.0 μM); $r = 0.412$.

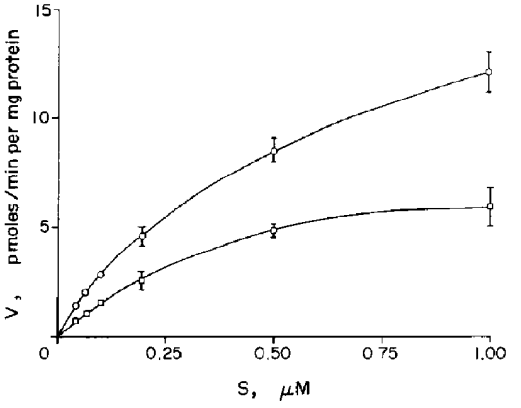

Fig. 3. The effect of mescaline on the uptake of noradrenaline. Plot of the temperature-dependent uptake against the NA concentration (as in Fig. 1). Open circles: NA control; $p^2 = 0.999$. Open squares: NA in the presence of 10 μM mescaline; $p^2 = 0.991$.

DISCUSSION

The results show that NA is accumulated into a synaptosome rich preparation from the rat cerebral cortex by an active uptake mechanism; The K_m for this uptake mechanism was 0.62 μM. This is in good agreement with previous reports; for example Wong, Horng and Fuller [14] found that the K_m for the active uptake of NA into synaptosomes was 0.63 μM. Similarly, Coyle and Snyder [8] reported that the K_m for NA uptake was 0.4 μM.

Desipramine competitively inhibited the uptake of NA in these experiments; the apparent K_m was increased whilst the V_m remained unchanged. In agreement with other authors (see [1]), the K_i for desipramine was found to be 0.049 μM. Shah and Himwich [15] reported that rat brain homogenates (whole brain less cerebellum) could accumulate small amouts

of mescaline, but concluded that the mechanism was probably a process of simple binding. This was later confirmed by Shah and Gulati [16] who were also unable to detect an active uptake process for mescaline.

The results presented in this paper show that a preparation from the rat cerebral cortex, rich in synaptosomes, may accumulate mescaline by a temperature- and sodium-dependent process. The K_m for this active process was found to be 1.24 μM. Thus, when compared with NA, the uptake process for mescaline is very weak.

Desipramine competitively inhibited the uptake of NA, but did not affect the uptake of mescaline. There was no change in the K_m or V_m for mescaline uptake when 0.05 μM desipramine was present in the incubation medium. Indeed, no effect of desipramine on mescaline uptake could be detected even when desipramine concentrations ranging from 0.05 to 5.0 μM were present in the incubation medium. In contrast, however, the binding of mescaline by rat brain homogenates was inhibited by high concentrations 1.33 m M) of desipramine [16.]

It would appear, then, that the NA uptake and mescaline uptake mechanisms could be different: the NA uptake mechanism is desipramine-sensitive, whereas the mescaline uptake mechanism is not. More evidence for a difference between NA and mescaline uptake processes is provided by the observation that the inhibition of NA uptake by mescaline is of a non-competitive nature. If NA and mescaline were accumulated by the same uptake mechanism one would expect mescaline to inhibit competitively the accumulation of NA.

It has recently been demonstrated that responses of single cortical neurones to mescaline can be potentiated by desipramine [5]. The results presented here, however, suggest that desipramine does not block the uptake of mescaline into cortical synaptosomes. We can conclude, therefore, that the potentiation by desipramine of neuronal responses to mescaline is not brought about by the blockade of mescaline uptake.

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