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EFFECTS OF THE CATECHOLAMINERGIC NEUROTOXIN *N*-(2-CHLOROETHYL)-*N*-ETHYL-2-BROMOBENZYLAMINE (DSP-4) ON ADRENAL CHROMAFFIN CELLS IN CULTURE*

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Abstract—*N*-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) is an inhibitor of noradrenaline uptake and a neurotoxin known to deplete noradrenaline levels with little effect on dopamine, serotonin or adrenaline in the central nervous system. The present study tested the effects of DSP-4 on catecholamine uptake, release and content in cultures of isolated bovine adrenal chromaffin cells. DSP-4 selectively inhibited the acute uptake of [^{3}H]noradrenaline with little or no effect on [^{3}H]adrenaline or [^{3}H]dopamine uptake. In cultures preloaded with [^{3}H]catecholamines, DSP-4 stimulated the release of [^{3}H]noradrenaline and, to a small extent, also [^{3}H]adrenaline and [^{3}H]dopamine. However, the drug did not stimulate the release of appreciable amounts of endogenous adrenaline, noradrenaline or dopamine. A high concentration of DSP-4 inhibited the carbachol-stimulated release of adrenaline, noradrenaline and dopamine from the cells. Following a 1-hr exposure to the drug, DSP-4 decreased adrenaline, noradrenaline and dopamine levels in the cells with no gross morphologic changes in the cells. Reductions in adrenaline and noradrenaline levels were almost equal in magnitude, while dopamine was depleted to a somewhat greater extent under some conditions. Longer exposure to DSP-4 resulted in morphological changes in the cells, suggesting that the drug is also toxic to chromaffin cells in culture.

N-(2-Chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4)‖ is a neurotoxin often used as a pharmacologic tool to chemically lesion noradrenergic pathways in the central nervous system [see, for example, Refs. 1–6]. The toxin produces long-lasting decreases in noradrenaline levels in most brain regions with little or no effect on levels of dopamine, serotonin or adrenaline [1, 2, 5–7]. In peripheral tissues, DSP-4 produces a more transient decrease in noradrenaline levels in tissues receiving a rich sympathetic innervation such as the heart, salivary glands and iris [1, 2]. Although the precise mechanism by which DSP-4 produces depletion of noradrenaline is not known, the toxin has been reported to have several effects on noradrenaline metabolism which may be related to its specific neurotoxicity. For example, DSP-4 and the DSP-4-related derivative, xylamine, have been shown to inhibit noradrenaline uptake into both central and peripheral noradrenergic tissues [7–13]; in brain slices and homogenates, DSP-4 and xylamine were found to be much more active in inhibiting uptake of noradrenaline compared to uptake of either dopamine or serotonin [7–9, 11]. The aziridinium derivative of DSP-4 (probably the reactive metabolite of DSP-4 [14, 15]) also has been reported to enhance the spontaneous release of noradrenaline from sliced cerebral cortex and isolated heart atria [16, 17].

In addition to the central nervous system and peripheral sympathetic nerves, high levels of catecholamines are also found in another cell type, the chromaffin cells of the adrenal medulla. Chromaffin cells are not, however, a homogeneous cell population. Adrenaline and noradrenaline are synthesized in separate chromaffin cells [see reviews in Refs. 18 and 19]. The exact proportion of the two catecholamines differs between various animal species [see, for example, Ref. 20], the ratio of adrenaline:noradrenaline being approximately 3:1 in the bovine adrenal gland [19–21, present study]. Dopamine, present at a level of about 1–2% of the total catecholamine content of the adrenal medulla [20, present study], may be stored in a distinct cell type, the "small granule-containing" cell [22–24]. Adrenal chromaffin cells are also known to contain numerous neuropeptides (e.g. Met- and Leu-enkephalins [25–27] and some of their larger molecular weight congeners [28], dynorphin [29], neurotensin [30, 31], substance P [32, 33], somatostatin [34], neuropeptide Y [35, 36], galanin¶, vaso-active intestinal peptide¶ [37, 38], atrial natiuretic peptide [39]), as well as small amounts of serotonin [40] and histamine [41]. Some of these neurohormones have been shown to co-localize with adrenaline¶ [26, 27, 29, 35, 36, 40], others with noradrenaline [29, 31, 41].

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‖ Abbreviations: A, adrenaline; DA, dopamine; DSP-4, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine; and NA, noradrenaline.

¶ Pruss RM, Rökeaus Å, O'Donohue TL and Eiden LE, Analysis of multiple neuropeptide-producing subpopulations of cultured bovine chromaffin cells. *Third International Symposium on Chromaffin Cell Biology*, Abstr. p. 61, 1986.

It has further been demonstrated that only certain subpopulations of adrenaline or noradrenaline cells may co-store a given neuropeptide [see, for example, Refs. 27 and 28], and even two chromaffin cells which both contain adrenaline and the same neuropeptide (e.g. vasoactive intestinal peptide) may differ markedly in their responses to agents that regulate neuropeptide levels in these cells [38]. Obviously then, a neurotoxin which might target a specific subpopulation of chromaffin cells would provide a very useful pharmacologic tool.

With this background in mind, the aims of the present study were: (1) to test the effects of DSP-4 on catecholamine uptake and release in chromaffin cells, (2) to determine whether DSP-4 is able to deplete catecholamines from adrenal chromaffin cells and whether this effect is selective for a specific catecholamine-containing cell type (i.e. adrenaline, noradrenaline or dopamine), and (3) to determine whether the selectivity of DSP-4 for depleting catecholamines might correlate with selective effects of the drug on catecholamine uptake or release in these cells. These experiments were carried out using cultures of chromaffin cells isolated from adult bovine adrenal glands.

MATERIALS AND METHODS

Materials. DSP-4 was purchased from Research Biochemicals Inc., Natick, MA, U.S.A.; for each experiment a fresh solution of DSP-4 was made up immediately before use. Levo-[*N-methyl*-^{3}H]adrenaline (78.1 Ci/mmol), levo-[7-^{3}H]noradrenaline (14.9 Ci/mmol) and 3,4-[8-^{3}H(N)]dihydroxyphenylethylamine (dopamine, 18.9 Ci/mmol) were purchased from New England Nuclear, Canada.

Chromaffin cell cultures. Chromaffin cells were isolated by retrograde perfusion of adult bovine adrenal medullae with DNAse 1 and collagenase and purified by density gradient centrifugation in Percoll as described by Trifaró *et al.* [42] and Trifaró and Lee [43]. Purified chromaffin cells were plated on collagen-coated plastic Multiwell plates (Falcon, 24 wells/plate) at a density of 2.5×10^5 cells/well and were maintained in culture for 5–14 days under conditions described previously [44].

[^{3}H]*Catecholamine uptake.* Each culture was washed three times with 0.5 ml of a Krebs–Ringer buffer and pre-equilibrated for at least 5 min in 0.5 ml buffer. The culture was then incubated at 23° with 0.5 ml buffer containing 10^{-3} M ascorbate and 10^{-7} M [^{3}H]adrenaline, [^{3}H]noradrenaline or [^{3}H]dopamine. The concentration of [^{3}H]catecholamines used is slightly less than the apparent K_m (3.5×10^{-7}–6.7×10^{-7} M) reported for uptake of [^{3}H]noradrenaline by adult bovine adrenal chromaffin cells in culture [45]. Immediately following this, each culture was rapidly washed five times with 0.75 ml of ice-cold buffer. [^{3}H]Catecholamines taken up by the cells were extracted by incubating the cells with 0.5 ml of acidic ethanol (95% ethanol : 5% 0.1 N HCl) for 0.5 hr, and radioactivity in an aliquot of the acidic ethanol extract was determined by liquid scintillation spectrometry. The composition of the standard Krebs–Ringer buffer used was as follows: NaCl (125 mM), KCl (4.8 mM), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES, 25 mM), $MgSO_4$ (1.2 mM), KH_2PO_4 (1.2 mM), glucose (5.6 mM) and $CaCl_2$ (2.2 mM).

Release of radiolabeled and endogenous catecholamines. In experiments measuring release of [^{3}H]catecholamines, chromaffin cell cultures were preloaded with 10^{-7} M [^{3}H]adrenaline, [^{3}H]noradrenaline or [^{3}H]dopamine as described in detail previously [46]. Immediately following loading, release of [^{3}H]catecholamines at 23° was measured as follows. Each culture was washed once with 0.5 ml buffer and was incubated for 5 min with buffer alone to determine basal release followed by another 5-min incubation with buffer plus 5×10^{-4} M carbachol to determine carbachol-stimulated release. When the effects of DSP-4 on basal and carbachol-stimulated release were tested, DSP-4 was present during both of the 5-min incubations described above. [^{3}H]Catecholamine release was measured by determining radioactivity in medium from release incubations. At the end of the experiment, each culture was extracted with acidic ethanol to determine residual cellular stores of radioactivity. [^{3}H]Catecholamine release was expressed as a percentage of the total [^{3}H]catecholamine stores for each culture to correct for variations in [^{3}H]catecholamine uptake between cultures.

Experiments measuring release of endogenous catecholamines were performed similarly except that cultures were not preloaded with [^{3}H]catecholamines, basal and carbachol-stimulated release periods were 10 min rather than 5 min, and endogenous catecholamines in release medium and remaining in the cells were measured by HPLC.

Depletion of endogenous catecholamines by DSP-4. Chromaffin cell cultures were incubated for the time periods indicated with DSP-4 in a medium consisting of 50% Dulbecco's modified Eagle's medium and 50% F12 nutrient medium. The cultures were maintained at 37° in a humid atmosphere gassed with 5% CO_2 in air, until their catecholamine content was extracted for measurement by HPLC.

HPLC determination of endogenous catecholamines. Catecholamines in cell cultures and release medium were extracted for quantification by HPLC according to methods modified from Müller and Unsicker [47]. Each culture was washed twice with Krebs–Ringer buffer. The cells were then scraped into 0.7 ml of 0.1 M perchloric acid containing 0.1 mM EDTA and 42 ng of epinine as internal standard, sonicated for 15 sec, and centrifuged (8000 *g*) for 5 min at 4°. An 0.5-ml aliquot of the supernatant (or 0.5 ml of release medium in release experiments) was mixed vigorously for 10 min with 10 mg of acid-washed Al_2O_3 and 0.8 ml of 1.5 M Tris–HCl (pH 8.6) containing 20 mM $NaHSO_3$ and 125 mM EDTA. The alumina was pelleted by a short low speed centrifugation and the supernatant was aspirated and discarded. The alumina was washed twice by vigorous mixing with 1.2 ml of 6 mM Tris–HCl (pH 8.6) containing 1 mM EDTA, centrifuging, and discarding the supernatant, followed by a third wash with 1.2 ml of 5 mM $NaHSO_3$. The final alumina pellet was resuspended in 0.2 ml of 0.1 M perchloric acid and allowed to stand for 15 min with intermittent vortexing to allow for extraction of the cat-

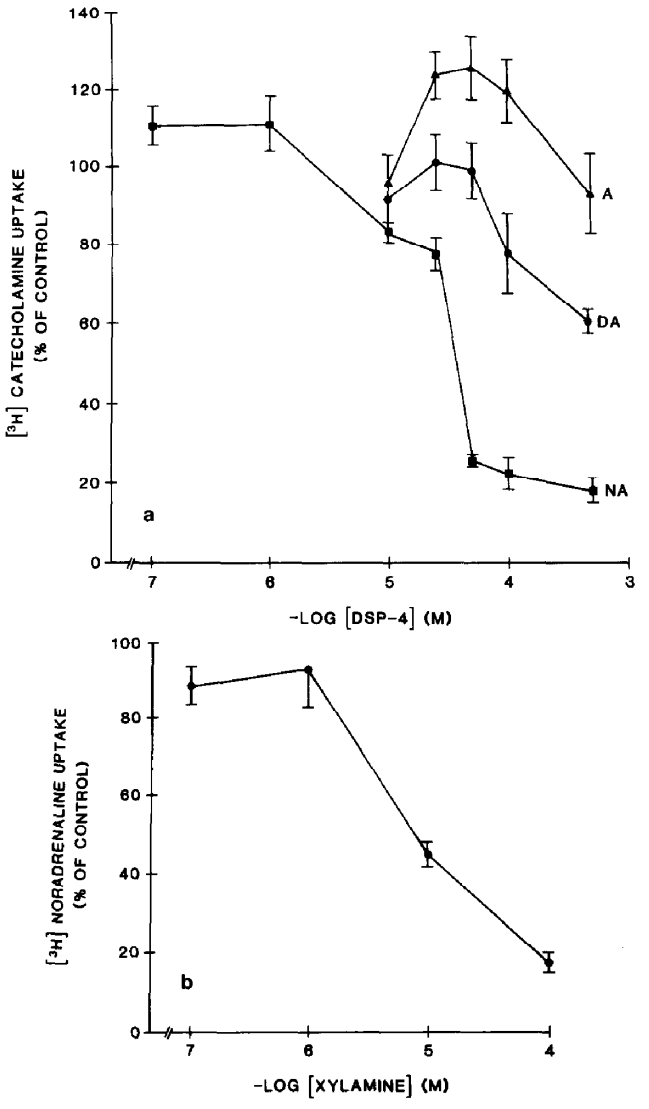

Fig. 1. Effects of DSP-4 (a) and xylamine (b) on [^{3}H]catecholamine uptake by chromaffin cell cultures. Chromaffin cell cultures were preincubated for 5 min with the indicated concentration of DSP-4 or xylamine. Following this, the cultures were incubated for 5 min with 10^{-7} M [^{3}H]adrenaline (A), [^{3}H]dopamine (DA) or [^{3}H]noradrenaline (NA) in the presence of the same concentration of DSP-4 or xylamine. Cultures were then washed rapidly with ice-cold buffer, and [^{3}H]catecholamine uptake by the cells was determined. Results are the means ± SEM from four to twelve cultures. Control uptake was 220.9 ± 17.0 fmol of [^{3}H]A, 342.8 ± 45.1 fmol of [^{3}H]DA and 444.2 ± 74.6 fmol of [^{3}H]NA per 5 min per 10^6 cells (means ± SEM from six to seven control cultures). Values significantly different from control in (a) are: for NA at DSP-4 concentrations of 10^{-5} M ($P < 0.05$), 2.5×10^{-5} M ($P < 0.025$), 5×10^{-5} M ($P < 0.0005$), 10^{-4} M ($P < 0.0005$) and 5×10^{-4} M ($P < 0.0005$) and for DA at 5×10^{-4} M DSP-4 ($P < 0.005$); in (b) for NA at xylamine concentrations of 10^{-5} M ($P < 0.005$) and 10^{-4} M ($P < 0.0005$).

echolamines from the alumina. Following another short low speed centrifugation, the supernatant was transferred to the HPLC. The HPLC conditions used for separation and detection of catecholamines are those described by Renner and Luine [48], except that a Novapak C18 column (3.9 × 15 cm, Waters Associates Inc.) was used; these conditions afforded good separation between adrenaline, noradrenaline and dopamine with a lower limit of detection of 10 pg.

Statistics. The significance of differences between groups was analyzed using a one-way analysis of variance with post-hoc *t*-tests.

RESULTS

[^{3}H]*Catecholamine uptake.* To test the acute effects of DSP-4 on [^{3}H]catecholamine uptake, chromaffin cell cultures were incubated for 5 min with 10^{-7} M [^{3}H]catecholamine in the presence of various

Table 1. Effects of DSP-4 on basal and carbachol-stimulated [^{3}H]catecholamine release from chromaffin cell cultures

[DSP-4]	[^{3}H]Catecholamine release (% of intracellular [^{3}H]catecholamine stores) [^{3}H]A	[^{3}H]NA	[^{3}H]DA
	Basal release		
0	2.5 ± 0.2	2.5 ± 0.4	4.2 ± 0.4
10^{-7}M	2.8 ± 0.3	3.8 ± 0.8	4.1 ± 0.6
10^{-6}M	3.3 ± 0.2*	2.6 ± 0.3	4.5 ± 0.3
10^{-5}M	3.0 ± 0.2	3.2 ± 0.4	5.1 ± 0.1†
10^{-4}M	3.2 ± 0.1‡	7.1 ± 1.1§	5.0 ± 0.7
	Carbachol-stimulated release		
0	8.5 ± 0.8	13.8 ± 0.5	11.4 ± 1.7
10^{-7}M	8.1 ± 0.5	14.1 ± 0.8	10.4 ± 0.3
10^{-6}M	9.4 ± 0.4	14.8 ± 0.6	10.5 ± 0.6
10^{-5}M	8.5 ± 0.8	13.0 ± 0.7	11.0 ± 0.3
10^{-4}M	8.0 ± 0.3	9.5 ± 1.2‡	9.2 ± 0.6

Chromaffin cell cultures were preloaded with [^{3}H]adrenaline (A), [^{3}H]noradrenaline (NA) or [^{3}H]dopamine (DA) as described in Materials and Methods. Cultures were then incubated for 5 min with buffer (for basal release) followed by 5 min with carbachol (5×10^{-4} M) in the presence of the indicated concentration of DSP-4, and ^{3}H released into the medium was measured. Results are means ± SEM from three to five cultures.

*–§ Significantly different from control: * $P < 0.05$, † $P < 0.025$, ‡ $P < 0.01$ and § $P < 0.005$.

Table 2. Effects of DSP-4 on basal and carbachol-stimulated release of endogenous catecholamines from chromaffin cell cultures

[DSP-4]	Catecholamine release (% of intracellular catecholamine stores) A	NA	DA
	Basal release		
0	1.8 ± 0.6	2.3 ± 0.2	2.9 ± 0.8
10^{-6}M	0.9 ± 0.2	1.4 ± 0.2*	2.1 ± 0.2
10^{-5}M	0.9 ± 0.1	1.7 ± 0.3	1.9 ± 0.2
10^{-4}M	1.3 ± 0.4	2.2 ± 0.4	2.0 ± 0.3
5×10^{-4}M	1.1 ± 0.2	1.8 ± 0.2	2.2 ± 0.5
	Carbachol-stimulated release		
0	13.1 ± 1.2	22.3 ± 3.6	18.1 ± 1.7
10^{-6}M	13.3 ± 0.4	24.1 ± 1.2	18.3 ± 0.6
10^{-5}M	12.6 ± 0.8	23.9 ± 0.8	17.7 ± 0.6
10^{-4}M	12.9 ± 1.1	23.7 ± 1.2	16.6 ± 0.5
5×10^{-4}M	5.2 ± 0.6†	10.5 ± 0.9†	7.9 ± 0.4†

Chromaffin cell cultures were incubated for 10 min with buffer (for basal release) followed by 10 min with carbachol (5×10^{-4} M) in the presence of the indicated concentrations of DSP-4, and endogenous adrenaline (A), noradrenaline (NA) and dopamine (DA) released into the medium were measured. Results are means ± SEM from three to five cultures.

*† Significantly different from control: * $P < 0.05$, and † $P < 0.005$.

concentrations of DSP-4, and [^{3}H]catecholamine uptake was measured (Fig. 1a). Control cultures took up 220.9 ± 17.0 fmol of [^{3}H]adrenaline, 444.2 ± 74.6 fmol of [^{3}H]noradrenaline and 342.8 ± 45.1 fmol of [^{3}H]dopamine per 5 min per 10^6 cells (means ± SEM from six to seven cultures). Although [^{3}H]catecholamine uptake was measured at only a single extracellular [^{3}H]catecholamine concentration (10^{-7} M), it is still noteworthy that the ratio of control [^{3}H]adrenaline:[^{3}H]noradrenaline:[^{3}H]dopamine uptake (≈1:2:1.5) was very different from the ratio of endogenous adrenaline:noradrenaline:dopamine content (≈1:0.35:0.04, see Fig. 2 and Table 3) of the cells. At concentrations of 10^{-5} M and greater, DSP-4 significantly inhibited [^{3}H]noradrenaline uptake. However, DSP-4 (10^{-5} to 5×10^{-4} M) had no effect on [^{3}H]adrenaline uptake, and [^{3}H]dopamine uptake was inhibited only by the highest concentration (5×10^{-4} M) of DSP-4 tested. Thus, in the concentration range of 5×10^{-5} to 10^{-4} M, DSP-4 produced a marked (75–82%) inhibition of [^{3}H]noradrenaline uptake with no effect on [^{3}H]adrenaline or [^{3}H]dopamine uptake. Xylamine (10^{-5} and 10^{-4} M) also produced a significant inhibition of [^{3}H]noradrenaline uptake (Fig. 1b).

Release of radiolabeled and endogenous catecholamines. To test the acute effects of DSP-4 on [^{3}H]catecholamine release, chromaffin cell cultures were pre-loaded with [^{3}H]catecholamine and, following this, basal and carbachol-stimulated release of [^{3}H]catecholamines were measured in the presence of DSP-4 (Table 1). DSP-4 (10^{-4} M) increased basal [^{3}H]noradrenaline release by 184% over control; in the concentration range of 10^{-6} to 10^{-4} M, DSP-4 produced small (21–32%) increases in [^{3}H]adrenaline or [^{3}H]dopamine release. At the highest concentration tested (10^{-4} M), DSP-4 significantly inhibited carbachol-stimulated [^{3}H]noradrenaline release, while DSP-4 (in a concentration range of 10^{-7} to 10^{-4} M) did not affect the carbachol-stimulated release of [^{3}H]adrenaline or of [^{3}H]dopamine.

To determine whether the above-mentioned effects of DSP-4 on release of radiolabeled catecholamines reflect effects of the drug on endogenous catecholamine release, further experiments tested the effects of DSP-4 on the release of endogenous catecholamines from chromaffin cell cultures (Table 2). In contrast to results obtained with the radiolabeled catecholamines, DSP-4 (in a concentration range from 10^{-6} to 5×10^{-4} M) did not stimulate the basal release of endogenous adrenaline, noradrenaline or dopamine. At 5×10^{-4} M, DSP-4 inhibited the carbachol-stimulated release of all three catecholamines by about 50–60%.

Endogenous catecholamine content and cell morphology. To test the effects of DSP-4 on the endogenous catecholamine content of chromaffin cells, bovine adrenal chromaffin cell cultures were incubated for 24 hr with various concentrations of DSP-4 and the catecholamine content of the cultures was measured subsequently (Fig. 2). DSP-4, at a concentration of 5×10^{-5} M, significantly reduced the content of adrenaline, noradrenaline and dopamine in the cultures; at this DSP-4 concentration, the depletions in adrenaline and noradrenaline content were almost identical in magnitude, and the depletion in dopamine content was somewhat greater than

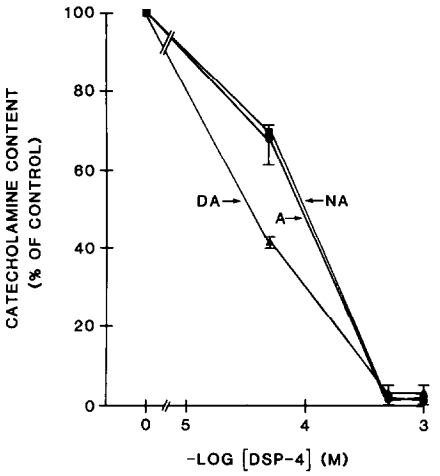

Fig. 2. Endogenous catecholamine content of chromaffin cell cultures treated with DSP-4 for 1 day. Chromaffin cell cultures were incubated for 24 hr with the indicated concentration of DSP-4; following this, cultures were extracted and assayed for their endogenous content of adrenaline (A), noradrenaline (NA) and dopamine (DA) by HPLC. Results are the means ± SEM from four to eight cultures treated with each concentration of DSP-4. Control cultures contained 11.37 ± 1.43 μg A, 4.08 ± 0.45 μg NA and 0.46 ± 0.07 μg DA per 10^6 cells (means ± SEM from nineteen to twenty control cultures). DSP-4 at the three concentrations tested produced significant reductions in the content of all three catecholamines ($P < 0.0005$ for all except A and NA at 5×10^{-5} M DSP-4 where $P < 0.025$); statistical analysis was performed on raw data before normalization to percent of control. With 5×10^{-5} M DSP-4, DA was depleted to a significantly greater extent than either A ($P < 0.005$) or NA ($P < 0.005$). At each of the other concentrations of DSP-4, there was no significant difference in the magnitude of depletion of the three catecholamines.

that of the other two catecholamines. With 5×10^{-4} M or 10^{-3} M DSP-4 there was almost a complete loss of all three catecholamines from the cultures.

In further experiments, cultures were incubated with 10^{-3} M DSP-4 for various time periods, and both the endogenous catecholamine content (Table 3) and the gross morphology of the cells were assessed. DSP-4 reduced adrenaline, noradrenaline and dopamine levels to about 30–40% of control values after a 1-hr treatment with the drug. However, there were no gross morphological changes evident in the cells after a 1-hr treatment. After 2 hr of DSP-4 treatment there was a further reduction in content of all three catecholamines with the reduction in dopamine content being slightly greater than the reduction in adrenaline or noradrenaline content. With 2 hr of DSP-4 treatment the chromaffin cells retracted their processes and appeared rounded and somewhat smaller than control cells. In cultures incubated with DSP-4 for 24 hr, there was almost a complete loss of all three catecholamines, and many of the cells had detached and were floating in the medium; of those cells that remained attached, most were rounded and very much reduced in size.

DISCUSSION

DSP-4 inhibited the uptake of [^{3}H]noradrenaline by chromaffin cells with an IC_{50} of about 5×10^{-5} M. This effect was selective in that [^{3}H]adrenaline uptake was not affected by DSP-4 (up to 5×10^{-4} M) and [^{3}H]dopamine uptake was inhibited only by the highest concentration (5×10^{-4} M) of DSP-4 tested. This result may be somewhat surprising in view of the observations of Kenigsberg and Trifaró [45] that the K_i values for noradrenaline and adrenaline for inhibition of [^{3}H]noradrenaline uptake into cultured bovine chromaffin cells are very similar; this suggests that this uptake mechanism may transport noradrenaline and adrenaline equally well. In addition, although dopamine accounted for less than 3% of the endogenous catecholamines in chromaffin cells in the present study, the cells transported nearly equal amounts of [^{3}H]dopamine, [^{3}H]noradrenaline and [^{3}H]adrenaline when incubated with a 10^{-7}M concentration of the [^{3}H]catecholamines; this also suggests that [^{3}H]dopamine uptake may not be exclusively into dopaminergic cells (although plausible alternative explanations for this discrepancy may certainly be made). Previous studies have shown that the DSP-4-related compound, xylamine, inhibits noradrenaline uptake into PC12 cells [13] (a cell line derived from an adrenal chomaffin cell tumour) and cultured superior cervical ganglia [12] (a catecholaminergic tissue sharing the same embryologic origin and many characteristics with adrenal chromaffin cells); however, neither of these studies reported the effects of the drug on catecholamine content or release in these tissues.

DSP-4 (up to 5×10^{-4} M) did not stimulate the release of endogenous adrenaline, noradrenaline or dopamine from chromaffin cell cultures. However, in cultures that had been preloaded with [^{3}H]catecholamines, higher concentrations (10^{-5}–10^{-4} M) of DSP-4 stimulated the release of [^{3}H]catecholamines, most markedly [^{3}H]noradrenaline. Thus it appears that the drug is able to preferentially release a small pool of catecholamines that have been taken up recently. In previous studies demonstrating a stimulatory effect of the aziridinium derivative of DSP-4 (az-DSP-4) on noradrenaline release from rat cerebral cortical slices [16] and atria [17], [^{3}H]noradrenaline release from tissue preloaded with the radiolabeled catecholamine was also the measure of release used. However, in the latter study with rat atria the observation that DSP-4 enhanced the rate of beating of the atria (an effect blocked by propranolol, desipramine and reserpine and enhanced by pargyline) strongly suggests that az-DSP-4 does release appreciable amounts of endogenous noradrenaline from sympathetic nerves innervating the heart. The only clear effect of DSP-4 on the release of endogenous catecholamines in chromaffin cells was a marked inhibition of the carbachol-stimulated release of adrenaline, noradrenaline and dopamine (by 5×10^{-4} M DSP-4).

The present study demonstrates that DSP-4 is able to deplete the catecholamine content of adrenal chromaffin cells. Following a 1-hr exposure of the cells to 10^{-3} M DSP-4, there was a significant depletion of the three catecholamines, adrenaline, noradrenaline

Table 3. Catecholamine content of chromaffin cell cultures treated with DSP-4 for various time periods

Time of DSP-4 treatment	Catecholamine content (% of control)		
	A	NA	DA
1 hr	40.1 ± 10.5	43.0 ± 10.3	31.9 ± 8.1
2 hr	30.3 ± 2.7	36.3 ± 3.0	18.5 ± 1.7
1 day	2.4 ± 0.7	1.9 ± 0.5	2.2 ± 0.7
2 days	0.9 ± 0.4	0.6 ± 0.02	0.7 ± 0.04

Chromaffin cell cultures were incubated with 10^{-3} M DSP-4 for the indicated time period and then were assayed for their endogenous content of adrenaline (A), noradrenaline (NA) and dopamine (DA). Results are means ± SEM from 4–8 cultures treated with DSP-4 for each time period. Control cultures contained 3.90 ± 0.50 μg A, 1.44 ± 0.13 μg NA and 0.14 ± 0.02 μg DA per 10^6 cells (means ± SEM from 24–25 control cultures). All values are statistically different from control (P at least <0.01); statistical analysis was performed on raw data before normalization to percent of control. At 2 hr, DA was depleted to a significantly greater extent than was either A ($P < 0.005$) of NA ($P < 0.0005$). At each of the other time periods, there was no significant difference in the magnitude of depletion of the three catecholamines.

and dopamine, from the cells with, however, no gross morphological changes in the cells. With longer exposure to the drug, there was further depletion of catecholamine content and the cells became rounded and reduced in size. After 24 hr of DSP-4 treatment, many of the cells had lifted off the culture plate, a situation indicative of cell death in these cultured cells. This suggests that DSP-4 is toxic to the cells in addition to depleting them of their catecholamine content. Since the chromaffin cells used in this study were cultured and isolated from their normal innervation, the catecholamine depleting and toxic effects of DSP-4 were due to a direct interaction of DSP-4 with chromaffin cells rather than to an indirect interaction of the drug with the nerves supplying the chromaffin cells.

With all treatment periods and concentrations of DSP-4 tested, there was no difference in the magnitude of depletion of the two catecholamines, adrenaline and noradrenaline, from chromaffin cells. DSP-4 did produce a somewhat preferential depletion of dopamine from the chromaffin cell cultures, in that depletion of dopamine was greater than that of adrenaline and noradrenaline with shorter treatment times and lower concentrations of DSP-4. This contrasts with the effects of the drug in brain, where DSP-4 has been shown to deplete noradrenaline with little effect on dopamine or adrenaline levels [1, 2, 5–7].

The slight selectivity of DSP-4 to deplete dopamine to a greater extent than adrenaline and noradrenaline from chromaffin cells (Fig. 2, Table 3) does not correlate with the drug's selective inhibition of acute [^{3}H]noradrenaline uptake. This suggests that an inhibition of catecholamine uptake may not be required for DSP-4 to deplete catecholamines from these cells. Alternatively, with longer exposure to DSP-4 the selectivity of the drug for inhibition of catecholamine uptake may be altered; however, this interpretation would require that, with a 5-min incubation, DSP-4 selectively inhibits [^{3}H]noradrenaline uptake while, with longer incubations, the drug inhibits uptake of all three catecholamines with a slight preference for dopamine. In addition, DSP-4 does not appear to deplete the catecholamine content of chromaffin cells solely by stimulating the release of the intact catecholamines into the extracellular medium since no release of catecholamines was measured following a 10-min incubation with DSP-4. A 10-min exposure to the drug should have released detectable amounts of the catecholamines if release were to account for the magnitude of depletion observed following a 1-hr exposure to the drug.

In summary, the catecholaminergic neurotoxin, DSP-4, was able to deplete catecholamines from adrenal chromaffin cells showing little selectivity for any one of the three catecholamines, adrenaline, noradrenaline or dopamine, and was probably neurotoxic to the cells. The drug also selectively inhibited [^{3}H]noradrenaline uptake but did not stimulate the release of endogenous adrenaline, noradrenaline or dopamine.

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