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# Monoamine Release by Compound 48/80 from Nonmast Cell Compartments in Mouse Brain Slices[1]

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## ABSTRACT

In the present study we investigated the specificity of the releasing effects of compound 48/80 (48/80) for mast cell *vs.* neuronal histamine (HA) from hypothalamic slices. In addition, we investigated the selectivity of the releasing effects of 48/80 for HA compared to other neurotransmitters. Brain slices from W/W mice, a genetic mutant devoid of mast cells, and +/+ mice, its normal counterpart, were used. Hypothalamic slices were labeled with [$^3$H]HA or [$^3$H]histidine. 48/80 elicited similar degrees of release of [$^3$H]HA from both mouse strains, irrespective of the label used. In addition, 48/80 produced marked increases in the efflux of dopamine (DA), serotonin (5-HT), norepinephrine (NE) and modest increases in the efflux of acetylcholine (ACh). These effects were concentration-dependent and the magnitude of release varied with the transmitter examined (DA > NE > 5-HT ≫ ACh = HA). Transmitter efflux induced by 48/80 was not altered by low calcium concentrations or by tetraethylammonium; whereas, release evoked by electrical stimulation was reduced and increased, respectively, by these treatments. In mouse striatal slices preloaded with [$^3$H]DA, 48/80 induced an initial increase in [$^3$H]DA efflux, followed by a marked increase in the efflux of its metabolite, [$^3$H]-3,4-dihydroxyphenylacetic acid. Nomifensine failed to inhibit, whereas reserpine pretreatment reduced 48/80-induced efflux of $^3$H. In summary, these results indicate that the incubation of hypothalamic slices with [$^3$H]HA or [$^3$H]histidine labels HA neurons selectively and that 48/80 (10–100 μg/ml) releases HA from nonmast cell compartments. In addition, 48/80 is specific neither for mast cells nor for HA neurons, as it can induce the release of DA, NE and 5-HT. 48/80 appears to enter the nerve terminals by passive diffusion affecting transmitter storage and binding which leads to increases in intraneuronal metabolism and efflux of unchanged as well as metabolized transmitter.

In recent years, there has been a resurgence in the interest of HA as a neurotransmitter (Schwartz *et al.*, 1980, 1986; Pollard and Schwartz, 1987). One way in which HA secretion from the brain has been studied is by the measurement of the release of [$^3$H]HA from brain slices. Two approaches to this method have been to study the release of exogenously added [$^3$H]HA or endogenously synthesized [$^3$H]HA from [$^3$H]histidine (Verdiere *et al.*, 1975; Subramanian and Mulder, 1976; Biggs and Johnson, 1980; Arrang *et al.*, 1983, 1985; Mulder *et al.*, 1983). Both techniques have been used successfully to characterize HA transmission in the central nervous system. However, a complication to the interpretation of these results has been the idea that mast cells may contribute to the uptake and release of the tritiated compounds which are incubated with the slices as well as be a source of release of endogenous HA. Goldschmidt *et al.* (1985) reported that mast cells contribute up to 50% of the total brain HA levels and as high as 90% in the thalamus. In order to delineate the origin of release of [$^3$H]HA, several investigators have used the drug 48/80 in concentrations ranging from 60 to 300 μg/ml (Subramanian and Mulder, 1976; Balfagon *et al.*, 1984). This drug (fig. 1) is a condensation product of *p*-methoxyphenethylmethylamine with formaldehyde. It is available as a mixture of short chain polymers (dimer-octamer) with the most HA-releasing activity associated with the hexamer (Read and Lenney, 1972). Its presumed primary mechanism of action is a degranulation of mast cells thereby releasing HA as well as other vesicular constituents of mast cells. Furthermore, 48/80 has been used with the notion that if it released HA then this HA must have originated from mast cells. However, recent studies indicate that 48/80 may release NE from cat cerebral arteries (Balfagon *et al.*, 1984). The present study was conducted to investigate the specificity of the effects of 48/80 on mast cell HA versus nonmast cell HA, and to determine whether this compound may induce release of other neurotransmitters. To achieve these goals, we used two strains of mice for our studies. One strain (W/W) is a genetic mutant which is devoid of mast cells (Yamatodani *et al.*, 1982), the other (+/+) is its normal genetic counterpart. The release of HA, 5-HT and NE from the hypo-

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**ABBREVIATIONS:** HA, histamine; 48/80, compound 48/80; NE, norepinephrine; 5-HT, serotonin; DA, dopamine; ACh, acetylcholine; DOPAC, 3,4-dihydroxyphenylacetic acid; RPU, reserpine, pargyline and U-0521; PU, pargyline and U-0521; TEA, tetraethylammonium.

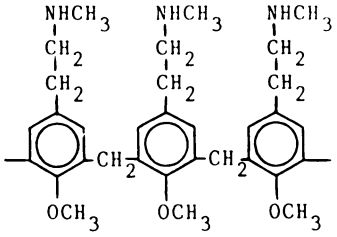

Fig. 1. Structure of the trimer of 48/80.

thalamus and the release of DA and ACh from the striatum were studied in these strains of mice. Particular emphasis was placed on the study of DA release from the striatum because it is well characterized and because 48/80 induced a large efflux of striatal DA and DA metabolites. Our results suggest new actions for 48/80 that are independent of mast cells.

## Methods

**Superfusion procedure.** Male mice, +/+ and W/W (Jackson Laboratories, Bar Harbor, ME), were used in all experiments. Mice were sacrificed by decapitation. Brains were removed quickly and hypothalamus and striatum were subsequently dissected on ice in a cold room (4°C). Tissue was chopped by a McIlwain Tissue Chopper into 0.4-mm slices (about 6 slices per hypothalamus). Slices were incubated at 37°C in 2 ml of superfusion medium. Time of incubation and concentration of radiolabeled transmitter depended on the compound used. ([$^3$H]DA, 30 min, 0.2 $\mu$M; [$^{14}$C]choline, 30 min, 5 $\mu$M; [$^3$H]HA, 30 min, 0.3 $\mu$M; [$^3$H]5-HT, 30 min, 0.1 $\mu$M; [$^3$H]NE, 30 min, 0.2 $\mu$M; and [$^3$H]histidine, 60 min, 0.5 $\mu$M). Composition of the superfusion medium was as follows (millimolar): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25; $KH_2PO_4$, 1.2; disodium EDTA, 0.03; ascorbic acid, 0.57; and glucose, 11. Medium was gassed continuously with 95% $O_2$-5% $CO_2$ to maintain a pH of 7.4.

After incubation, slices were washed 4 times in twice the incubation volume of warm superfusion medium. Two slices were then transferred to each of 12 polypropylene cylinders in which they were supported by a piece of nylon mesh. The cylinders were placed in glass superfusion chambers and positioned between two platinum electrodes. The slices were then superfused with prewarmed superfusion medium (37°C) at a rate of 1 ml/min. Sample collection began at different times depending on the tritiated compound used. Five-minute samples were collected. Experiments involving [$^{14}$C]ACh were superfused in the presence of hemicholinium-3 (10 $\mu$M) in order to prevent [$^{14}$C]choline reuptake. In experiments involving stimulated transmitter release, electrical stimulation was achieved with trains of unipolar, rectangular pulses (2 msec, 10 V) at a frequency of 5 Hz, 600 pulses for NE, 5-HT and HA and at 1 Hz, 120 pulses to induce DA and ACh release. Newly synthesized [$^3$H]HA was separated from [$^3$H]histidine over DOWEX ion exchange resin according to a modified version of the method of van der Werf *et al.* (1987). Radioactivity in the superfusate and in the tissue slices was determined by liquid scintillation counting. Basal $^3$H-release was expressed as a fractional rate of loss from the tissue: (radioactivity in 5 min superfusate fraction/total radioactivity in slice at the beginning of the fraction collection).

Separation of DA metabolites was performed according to Cubeddu *et al.* (1979). Briefly, 10-ml superfusate samples were collected and acidified with 375 $\mu$l of 0.2 N HCl containing 2.85 mg of $Na_2S_2O_5$, 1.1 mg of disodium EDTA and 10 $\mu$g each of DA and DOPAC. Fractions were then adjusted to pH 8.3 with 3 ml of an ice-cold 1.5 M Tris HCl-2% disodium EDTA (pH 8.6) solution. Samples were then added to columns containing 200 mg of alumina and subsequently eluted with 1 ml of water, 4 × 0.5 ml of acetic acid (0.1 N), and 3 × 0.5 ml of HCl (0.6 N) for HVA, DA and DOPAC, respectively.

In some experiments, mice were pretreated with reserpine (5 mg/kg) 24 hr before sacrifice. The vehicle was 0.17 M acetic acid in saline-ethanol (1:1). In order to achieve labeling of DA terminals, slices from the reserpinized animals were incubated in the presence of an inhibitor of monoamine oxidase B (pargyline, 0.5 mM) and an inhibitor of catechol-O-methyltransferase (U-0521, 30 $\mu$M); RPU conditions). Control slices (from vehicle injected animals) were also incubated in the presence of PU conditions.

**Materials.** All tritiated compounds were purchased from New England Nuclear (Boston, MA). Their specific activities were as follows: [$^3$H]DA, 29.5 Ci/mmol; [$^3$H]5-HT, 28.3 Ci/mmol; [$^3$H]NE, 14.9 Ci/mmol; [$^{14}$C]choline, 52 mCi/mmol; [$^3$H]HA, 31.7 Ci/mmol; and [$^3$H]histidine, 51.0 Ci/mmol. Other substances used in this study and their respective sources: 48/80 and hemicholinium-3 (Sigma Chemical Co., St. Louis, Mo.); nomifensine maleate (Hoechst-Roussel, Somerville, NJ); reserpine (Serpasil, Ciba-Geigy, Summit, NJ); and alumina (aluminum oxide 90, Art. 1097; Merck-AG, Rahway, NJ).

## Results

**Effects of 48/80 on the basal efflux of HA, DA, NE, 5-HT and ACh from +/+ and W/W mouse brain slices.** Figures 2 and 3 demonstrate that outflow of various monoamine transmitters from brain slices can be enhanced by either electrical stimulation or by exposure to 48/80. In all cases, increase in outflow of transmitter by either treatment is independent of the presence of mast cells as similar release was observed in slices from +/+ and W/W mice for both electrical stimulation and 48/80 exposure. 48/80 produced a concentration (10–100 $\mu$g/ml)-dependent increase in the basal efflux of radioactivity from slices preloaded with radioactive DA, NE, 5-HT, ACh or HA (figs. 2–4). However, the magnitude of efflux due to 48/80 varied depending on the transmitter examined (DA > NE > 5-HT > ACh = HA; fig. 4). For example, 48/80 (100 $\mu$g/ml) increased [$^3$H]DA efflux 5-fold; whereas [$^3$H]HA efflux from hypothalamic slices preloaded with [$^3$H]HA increased by 50%.

**Release of transmitters due to 48/80 is not $Ca^{++}$-dependent.** In order to determine the mechanism of action of 48/80, we investigated whether the increase in transmitter efflux induced by 48/80 was dependent on the concentration of extracellular $Ca^{++}$. Figure 5 shows that when the $Ca^{++}$ concentration of the superfusion medium was reduced from 1.3 to 0.13 mM, electrically stimulated release of [$^3$H]DA was abolished whereas release due to 48/80 remained unchanged. Similar results were obtained for the release of [$^3$H]5-HT, [$^3$H]NE, [$^3$H]HA and endogenously synthesized [$^3$H]HA (data not shown). Although results are shown only for W/W mice, similar findings were obtained with +/+ mice.

**Release of transmitter due to 48/80 is not blocked by a neuronal uptake inhibitor.** To test whether 48/80 could be eliciting transmitter release by an amphetamine-like mechanism (Parker and Cubeddu, 1986a,b), we tested its effects in the presence of a neuronal uptake inhibitor. The inhibitor nomifensine (3 $\mu$M) was used because it blocks the uptake of both NE and DA (Hunt *et al.*, 1974). Nomifensine (added 25 min before exposure to 48/80) failed to inhibit the release of either [$^3$H]DA from control as well as from reserpine-treated (RPU) striatal slices (fig. 6) or [$^3$H]NE from hypothalamic slices induced by 48/80. 48/80 increased [$^3$H]NE efflux by 4.2-fold in the absence and 3.9-fold in the presence of nomifensine ($P > .1$).

**Effects of reserpine pretreatment on transmitter release due to 48/80.** In order to determine a possible pool of transmitter which is sensitive to release by 48/80, we depleted mice of their vesicular stores of catecholamines. This was achieved by pretreatment of the mice with reserpine (5 mg/kg)

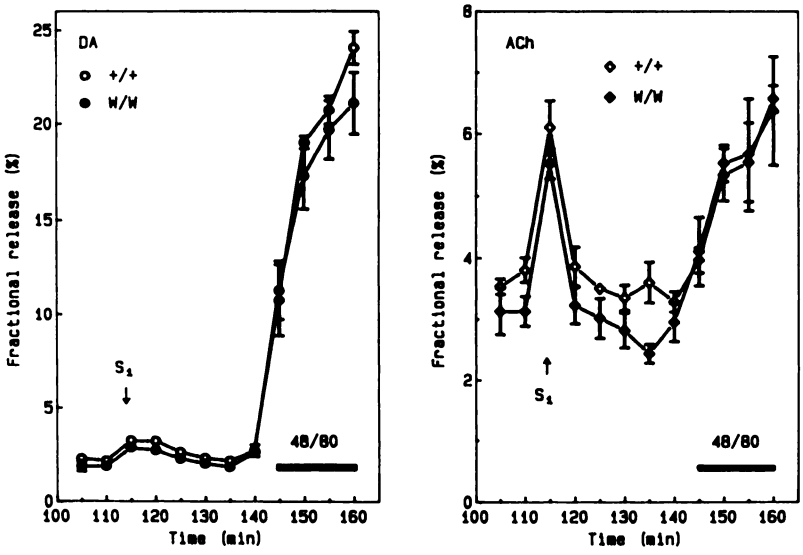


Fig. 2. Release of DA and ACh from mouse striatum induced by electrical stimulation and 48/80. Striatal slices from either +/+ or W/W mice were incubated with [$^3$H]DA and [$^{14}$C]choline then superfused. The slices were stimulated once ($S_1$; 1 Hz, 2 min, 120 pulses) 115 min after the beginning of superfusion. 48/80 (100 μg/ml) was added to the superfusion medium 25 min after electrical stimulation, when the efflux of radioactivity had returned to base-line levels. Results are expressed as the fractional release of radioactivity per 5-min sample. ○ ◇, +/+ slices; ● ◆, W/W slices. Shown are mean values ± S.E.M. ($n$ = 3).

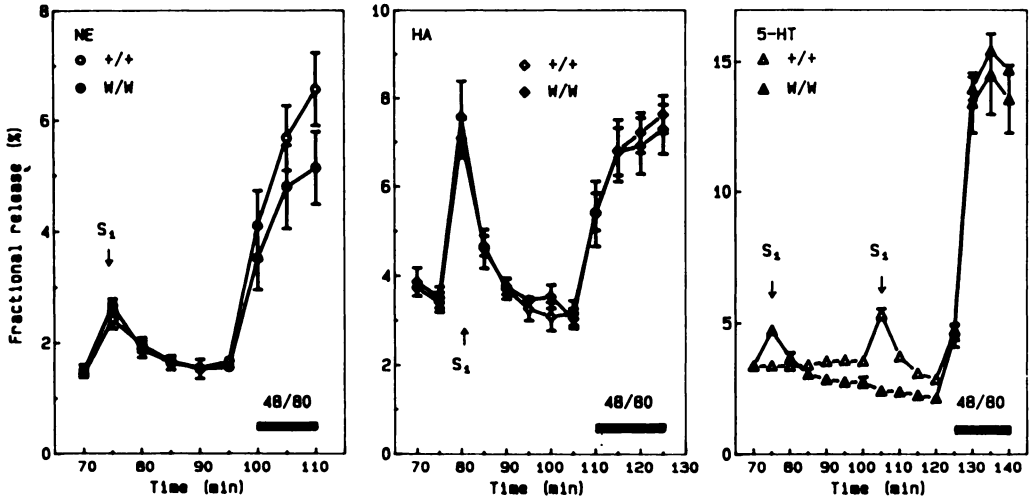


Fig. 3. Release of NE, HA and 5-HT from mouse hypothalamus induced by electrical stimulation and compound 48/80. Hypothalamus slices from either +/+ or W/W mice were incubated with [$^3$H]NE, [$^3$H]HA, or [$^3$H]5-HT and then superfused. The slices were stimulated once ($S_1$ at 5 Hz for 2 min (600 pulses). 48/80 (100 μg/ml) was added to the superfusion medium once the overflow of radioactivity returned to base-line levels. Results are expressed as the fractional release of radioactivity per 5-min sample. ○ ◇ △, +/+ slices; ● ◆ ▲, W/W slices. Shown are mean values ± S.E.M. ($n$ = 3).

for 24 hr before sacrifice. Brain slices from vehicle mice and from mice treated with reserpine were labeled with [$^3$H]DA and [$^{14}$C]choline. Slices from reserpine-treated mice were always incubated and superfused in the presence of pargyline and U-0521 to inhibit DA metabolism and to achieve neuronal labeling (RPU slices). Slices from control mice were incubated and superfused in the absence (untreated) or presence of PU. 48/80 induced a greater increase in $^3$H-efflux from control untreated slices than from reserpine treated (RPU) slices both in the presence and absence of nomifensine (fig. 6). Inhibition of DA metabolism by PU also reduced the basal efflux of total $^3$H in control slices (PU conditions); however, a similar fold increase in the efflux of total $^3$H was induced by 48/80 from control (untreated) and from monoamine oxidase, catechol-O-methyltransferase inhibited slices (PU) (fig. 6). This increase was greater than that observed in reserpine-treated slices. Pretreatment with reserpine had no effect on 48/80-induced increase in [$^{14}$C]ACh efflux (data not shown).

**Effects of 48/80 on the release of DA metabolites.** To understand the mechanism of action of 48/80 better, the metabolism of DA released by this drug was studied in the absence and presence of nomifensine (fig. 7). Under both conditions, 48/80 induced an increase in the efflux of [$^3$H]DA, [$^3$H]DOPAC and [$^3$H]HVA. However, the pattern of metabolism varied with the time of exposure to 48/80. Initially (first 10 min), [$^3$H]DA was the major fraction released, subsequently [$^3$H]DA release declined and [$^3$H]DOPAC release increased. Although the fractional rate of [$^3$H]HVA efflux was increased by 48/80, the proportion of the total radioactivity released as HVA did not change throughout the period of exposure to 48/80 (40 min). Although a similar time course for the efflux of DA and its metabolites induced by 48/80 was observed in the presence of nomifensine, a greater proportion of DA and a lower proportion of DOPAC were released by 48/80 in the presence of the uptake inhibitor (fig. 7).

**Release due to 48/80 is unaffected by a $K^+$ channel blocker.** 48/80 has been shown to elicit $K^+$ release from liposome preparations (Katsu *et al.*, 1983). Consequently we investigated whether blockade of $K^+$ channels with TEA could interfere with the releasing action of 48/80. Stimulation-evoked release of [$^3$H]5-HT from hypothalamic slices and of [$^3$H]DA from striatal slices was enhanced markedly in the presence of 10 mM TEA. However, TEA had no effect on 48/80-induced release of [$^3$H]5-HT (table 1) or [$^3$H]DA (not shown).

## Discussion

48/80 is known as a potent and "selective" mast cell degranulator (Johnson and Moran, 1969; Paton, 1951). Accordingly,

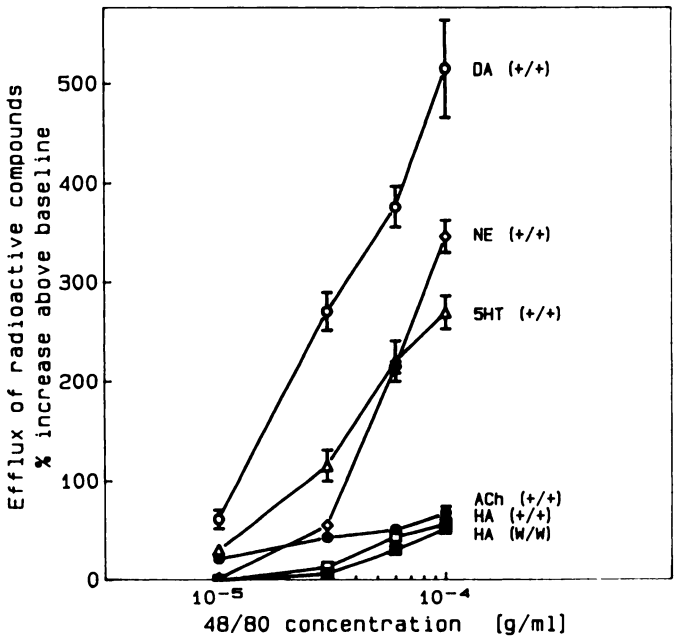


**Fig. 4.** Neurotransmitter release induced by 48/80. Dose-dependent effects of 48/80 are plotted as the percentage of increase in fractional rate of release induced by 48/80 [(48/80 induced fractional rate of release/basal fractional rate) × 100]. Striatal (DA and ACh) or hypothalamic slices (NE, 5-HT and HA) were incubated with radiolabeled transmittters and then superfused. Increasing doses of 48/80 (10, 30, 60 and 100 μg/ml) were added sequentially to slices after each concentration was perfused for 25 min ($n$ = 3).

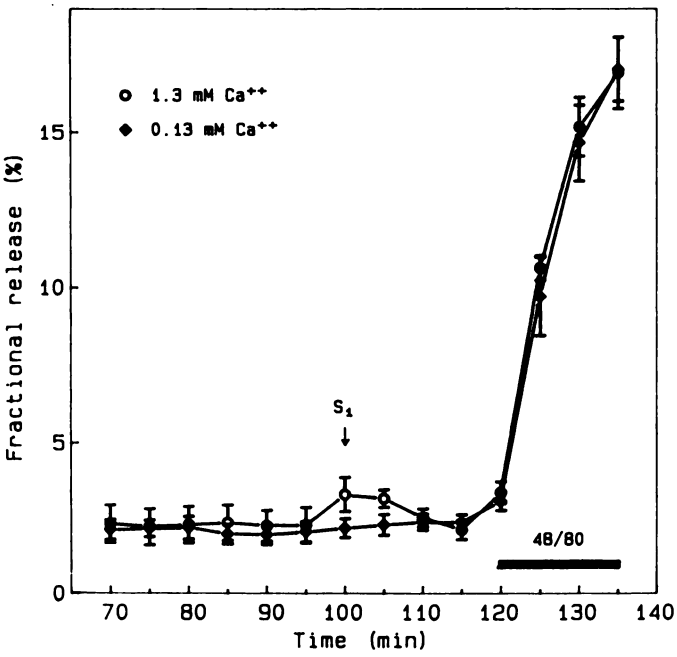


**Fig. 5.** DA release from mouse striatum induced by electrical stimulation and 48/80: calcium dependence. Striatal slices from W/W mice were incubated with [$^3$H]DA and then superfused. In some of the chambers, the superfusion medium was replaced with a low $Ca^{++}$ medium (0.13 mM $Ca^{++}$) after 70 min of superfusion. All of the slices were stimulated once ($S_1$; 1 Hz, 120 pulses) 95 min after the beginning of superfusion. 48/80 (100 μg/ml) was added to the superfusion medium after 115 min. Results are expressed as the fractional release of radioactivity per 5-min sample. O, slices in control medium; ◆, slices which were superfused with low $Ca^{++}$. Shown are mean values ± S.E.M. ($n$ = 3).

48/80 should release HA from mast cells and not from neuronal stores. Concentrations of 1 μg/ml up to 300 μg/ml of 48/80 have been used to elicit HA release from mast cells (Subramanian and Mulder, 1976; Balfagon *et al.*, 1984). However, data from this study indicates that 48/80 (at concentrations between 10–100 μg/ml) induces the release of HA from nonmast cell (most likely neuronal) sources. In addition, when tissues were prelabeled with radioactive transmitters or precursors, 48/80 at concentrations of 10 μg/ml or greater induced monoamine transmitter release. Therefore, it appears that 48/80 is not a selective mast cell degranulator.

Our major interest is to study the mechanisms of release of neuronal HA from the central nervous system and the contributions of newly synthesized and of stored HA to the released transmitter. However, these studies are complicated by the presence of mast cells in the central nervous system. Mast cells synthesize, store and release HA (Johnson and Moran, 1969; Thon and Unvas, 1966). Based on the concept that 48/80 is a selective mast cell degranulator, we used this compound to determine what proportion of the [$^3$H]HA (after incubation with [$^3$H]histidine or [$^3$H]HA) was in the mast cells. If 48/80 releases HA only from mast cells, then increases in [$^3$H]HA efflux should not occur from mast cell-deficient slices. To our surprise, 48/80 elicited similar increases in [$^3$H]HA release from hypothalamic slices of normal (+/+) and of the mast cell-deficient (W/W) mice. These results suggest that 48/80 releases [$^3$H]HA from nonmast cell compartments. Inasmuch as [$^3$H]HA from either incubation with [$^3$H]histidine or after labeling with [$^3$H]HA was released by electrical stimulation and this release was inhibited by low $Ca^{++}$, the source of this [$^3$H]HA release is most likely of neuronal origin. Furthermore, the results with [$^3$H]histidine incubation support this hypothesis. 48/80 failed to increase the release of [$^3$H]histidine whereas it increased the release of newly synthesized [$^3$H]HA from the same slices (data was not shown). Our results suggest that incubation of mouse hypothalamic slices with [$^3$H]HA or [$^3$H]histidine appears to label HA neurons and that 48/80 may release [$^3$H]HA from nonmast cell compartments. Recently, Balfagon *et al.* (1984) reported that 48/80 could release NE from cat cerebral arteries. These observations, together with our findings on HA release, led us to test whether 48/80 could induce neuronal release of transmitters, for which there is better evidence that labeling with radioactive transmitters is selective for neurons. We found that 48/80 enhanced the efflux of DA and ACh from corpus striatum and 5-HT, HA and NE from the hypothalamus. However, the magnitude of release induced by 48/80 varied depending on the transmitter examined (DA > NE > 5-HT > ACh = HA; fig. 4).

48/80 is a condensation product of *P*-methoxyphenethylmethylamine and formaldehyde. Tetrahydroisoquinolines are compound formed by the condensation of catecholamines with aldehydes (Schopf and Bayerle, 1934). Tetrahydroisoquinolines are known to accumulate in central noradrenergic and dopaminergic neurons, to induce DA and NE release, to inhibit neuronal transmitter uptake and to affect catecholamine metabolism (Heikkila *et al.*, 1971; Cohen *et al.*, 1972; Melchior *et al.*, 1978; Hoffman and Cubeddu, 1982). Therefore, because of the structural similarities between 48/80 and the isoquinolines, it is possible that 48/80-induced increases in transmitter release resemble those reported for the isoquinolines (*i.e.*, noncalcium-dependent transmitter release). A series of experiments were conducted to investigate the mechanism by which 48/80 in-

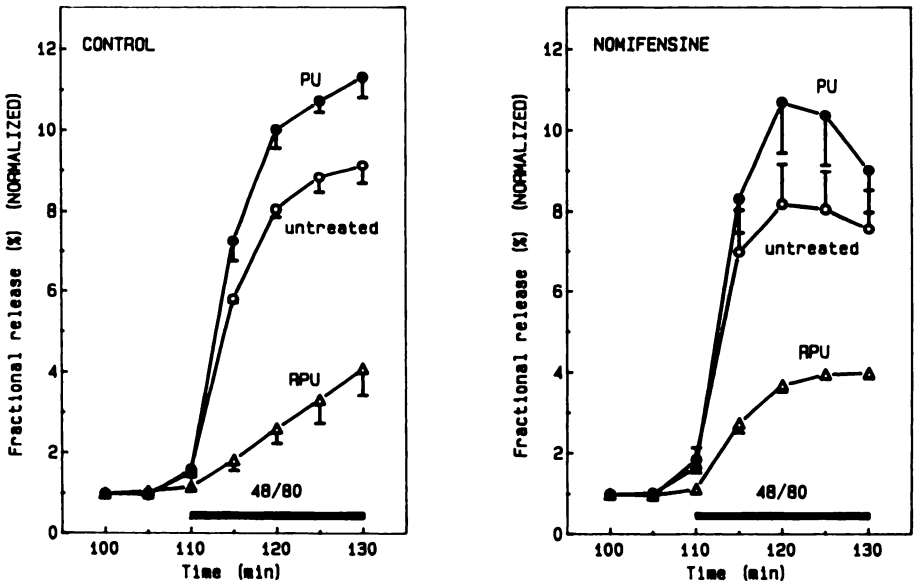


Fig. 6. 48/80-induced DA release from mouse striatum: effects of reserpine and of inhibition of metabolism. W/W mice were injected s.c. with reserpine (5 mg/kg) 24 hr before sacrifice. Control W/W mice were injected with vehicle (5 μl/g of a 0.17 M acetic acid in 1:1 saline-ethanol solution). Striatal slices from both groups of mice were incubated with [$^3$H]DA. In some experiments, slices were incubated and superfused with pargyline and U-0521 to inhibit DA metabolism. 48/80 (100 μg/ml) was added after 110 min of superfusion. Where present, nomifensine (3 μM) was added to the superfusion media 25 min before 48/80. Results are expressed as the normalized fractional release (actual fractional release/basal fractional release) of radioactivity per 5-min sample. ○, untreated slices; ●, control slices that were treated with PU; and △, slices from mice pretreated with reserpine (RPU) and incubated with RPU. Shown are mean values ± S.E.M. ($n$ = 3).

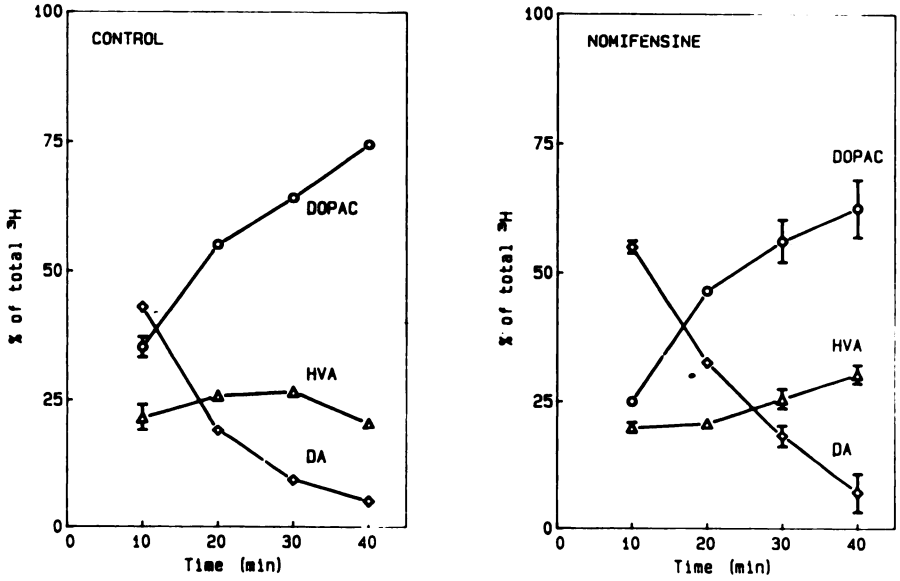


Fig. 7. Release of DA and its metabolites from mouse striatum after exposure to 48/80. Slices of +/+ mouse striatum were incubated with [$^3$H] DA and then superfused. Some of these slices were exposed to nomifensine (3 μM) after 30 min of superfusion and continued to the end of the experiment. Superfusion with 48/80 (100 μg/ml) started at 90 min of superfusion. Ten-minute fractions were collected and then run over alumina columns as described under "Methods." Three fractions of eluate were collected which represented homovanillic acid (HVA), DA and DOPAC, respectively. Results are graphed for each metabolite as percentage of distribution in the increase in $^3$H-efflux induced by 48/80. ○, DOPAC; △, HVA; and ◇, DA ($n$ = 3).

duced the release of neurotransmitters. The efflux induced by 48/80 was not blocked by low extracellular $Ca^{++}$, or was it enhanced by TEA, which suggests that the release is not $Ca^{++}$-dependent exocytotic release, or is it due to propagation of action potentials as TEA should have induced facilitation of release. Subsequently, we investigated whether 48/80 could be entering nerve terminals through the neuronal uptake carrier and causing the efflux of these transmitters, through a mechanism similar to that proposed for amphetamine (Parker and Cubeddu, 1986a,b). In order to delineate this possibility, we measured the efflux of [$^3$H]DA and of [$^3$H]NE induced by 48/80 in the presence of the neuronal uptake inhibitor nomifensine. Under these conditions, nomifensine failed to inhibit 48/80 induced DA and NE release. These results suggested that inward neuronal transport of 48/80 is not essential for the drug action, indicating that 48/80 could reach the intraneuronal transmitter pools by passive diffusion through the terminal membrane or through another transport process. The metabolite pattern of the increase in $^3$H-efflux induced by 48/80 was also different from that induced by amphetamine. The latter drug increased the efflux of DA and reduced that of DOPAC (Parker and Cubeddu, 1986a,b); whereas 48/80 increased the efflux of both DA and DOPAC. All these results suggest that 48/80 increases the cytoplasmic concentration of DA, some of which leaks out of the terminal as unchanged DA and the rest is metabolized inside the terminal and leaves as [$^3$H]DOPAC. The modest increase in DA efflux and the reduction in DOPAC efflux, observed when 48/80 was given in the presence of nomifensine, indicates that a small fraction of the DA released by 48/80 is recaptured by the DA neuronal transport. In addition, the results suggest that 48/80 was not blocking the neuronal uptake because of its ability to enhance DA and DOPAC efflux even in the presence of nomifensine.

Although neuronal storage vesicles may be the direct targets of 48/80, our data suggest that 48/80 could also release DA from reserpine-insensitive pools. Experiments conducted on control and reserpine-treated mice indicate that although the magnitude of $^3$H-efflux induced by 48/80 was greater from control-untreated slices than from reserpine-treated slices, 48/80 still induced significant efflux from reserpine-treated slices.

TABLE 1

**Effects of TEA on 48/80-induced 5-HT release and on the electrically evoked release of 5-HT from hypothalamus slices**

Hypothalamus slices from +/+ mice were prelabeled with [$^3$H]-5-HT. In some experiments (A), 48/80 (100 μg/ml) was added to untreated control slices or to slices exposed to 10 mM TEA. Shown are the fractional rates of $^3$H-efflux before (no drug) and 10 min after exposure to 100 μg/ml of 48/80. In additional experiments (B), the effects of TEA on the electrically evoked release of 5-HT were compared to those of control untreated slices. Slices were stimulated at 5 Hz for 2 min (600 pulses), after 70 min ($S_1$) and 120 min ($S_2$) of superfusion. When present, TEA (10 mM) was added 25 min before $S_2$. Stimulation (S) values = percentage of total $^3$H released by stimulation above base-line levels. Shown are mean values ± S.E.M.

| | Basal Efflux | | |
|---|---|---|---|
| | No drug | 48/80 | |
| A | | | |
| Control | 2.15 ± 0.05 | 13.41 ± 1.18 | |
| TEA | 3.51 ± 0.29 | 13.42 ± 0.43 | |
| | Stimulation-Evoked Overflow | | |
| | $S_1$ | $S_2$ | $S_2/S_1$ |
| B | | | |
| Control | 0.91 ± 0.22 | 0.90 ± 0.25 | 1.03 ± 0.27 |
| TEA | 0.86 ± 0.22 | 5.46 ± 0.50*** | 6.86 ± 1.05*** |

*** $P < .001$.

Thus, it appears that 48/80 is not only releasing DA from neuronal storage vesicles, but also "mobilizing" DA from the cytoplasm or membrane binding sites also known as extravesicular DA pools (see Parker and Cubeddu, 1986a,b for discussion). Because of the basic nature of 48/80, high intraneuronal or intravesicular concentrations of 48/80 can dissipate the transvesicular pH gradient and liberate vesicular DA into the cytoplasm. Such an effect had been observed with high concentrations of amphetamine (Philips, 1982; Johnson *et al.*, 1982). Alkalinization of the cytoplasm may also occur increasing the proportion of unionized, more lipophilic, DA which may leave the terminals in favor of a concentration gradient.

Inasmuch as we have studied the effects of 48/80 on DA release to a greater extent than on release of other transmitters, it is difficult to extrapolate the above-mentioned hypothesis to them. As shown in figure 4, 48/80 elicited much greater efflux of DA, NE and 5-HT than of ACh and HA. The reason for this selectivity is unknown. However, differences in the types and sizes of neuronal pools available for release and greater structural similarities existing between 48/80 and DA, NE and 5-HT molecules may allow 48/80 to more selectively recognize and displace these monoamines from their binding sites.

With regard to mast cells, the mechanism of action of 48/80 is not understood clearly. Studies performed on mast cells with spin-labeled 48/80 are consistent with the notion that 48/80 binds to integral membrane proteins of mast cells and is probably not in contact with the extracellular aqueous environment (Ortner and Chignell, 1980a,b). Hino *et al.* (1977) found that 48/80 covalently bound to Sepharose was still active. This supports the argument that the site of 48/80 action could be at the external membrane. However, if 48/80 does elicit its effects through binding on the terminal membrane, the mechanism by which it causes the release of transmitters does not appear to be due to changes in membrane depolarization. The inability of nomifensine to block transmitter efflux induced by 48/80 could indicate that the drug may act outside on the terminal membrane; but does not exclude the possibility that 48/80 enters the terminal through passive diffusion and displaces the transmitters from their binding sites leading to intraneuronal metabolism and efflux. Katsu *et al.* (1983) reported that 48/80 caused an increase in potassium efflux from a negatively charged liposome suspension, whereas it was ineffective in a positively charged liposome suspension. They suggested that perhaps insertion of a highly ionized polymer into the lipid bilayer may cause disruption of the lipid arrangement and thus result in potassium efflux. We found that the $K^+$ channel blocker TEA had no effect on 48/80-induced release of 5-HT or DA. However, there are more than one species of $K^+$ channels some of which are not sensitive to TEA. Interestingly, the changes in transmitter efflux induced by 48/80 resemble those induced by substitution of extracellular chloride ions with other impermeable anions (Diliberto *et al.*, 1987). Whether 48/80 acts on the membrane to affect the same changes induced by reducing the extracellular concentration of $Cl^-$ is unknown.

In summary, at concentrations greater than 10 μg/ml, 48/80 increases [$^3$H]HA efflux from hypothalamic slices of normal (+/+) and mast cell-deficient (W/W) mice which indicates that these concentrations of 48/80 release HA from nonmast cell compartments. Similarly, 48/80 enhances the efflux of DA, 5-HT, NE and ACh from mouse brain slices. It appears that the drug enters the nerve terminals, releases the transmitter from its vesicular and cytoplasmic storage and binding sites, increasing the efflux and intraneuronal metabolism of the transmitter. These results indicate that compound 48/80 is not a selective mast cell degranulating agent.

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