ELSEVIER

European Journal of Pharmacology 373 (1999) 163–169

ejp

www.elsevier.nl/locate/ejphar

Cu^{2+} induces Ca^{2+}-dependent neurotransmitter release from brain catecholaminergic nerve terminals

James K.T. Wang *

Departments of Neuroscience and of Physiology, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111, USA

Received 15 February 1999; received in revised form 7 April 1999; accepted 13 April 1999

Abstract

$CuCl_2$, $ZnCl_2$ and $NiCl_2$, but not $CdCl_2$ or $CoCl_2$, induced transmitter release from superfused rat hippocampal and striatal synaptosomes preloaded with, respectively, [^{3}H]noradrenaline and [^{3}H]dopamine. Cu^{2+} was the most potent and effective, acting in a concentration- (0.1–300 μM) and time-dependent (peak effect occurring at 2–3 min) manner. The amount of Cu^{2+}-induced release over a 5 min period is similar to that induced by depolarization with high KCl or the K^+ channel blocker 4-aminopyridine. However, the time course of the Cu^{2+}-induced release is slower and the effect of Cu^{2+} is not reversed by washout. Cu^{2+}-induced catecholamine release requires extracellular calcium (Ca^{2+}) and is inhibited by the Ca^{2+} channel blocker Cd^{2+}, and in the case of noradrenaline, by the voltage-gated Na^+ channel blocker tetrodotoxin. The ability of Cu^{2+} to induce massive Ca^{2+}-dependent transmitter release from brain catecholaminergic nerve terminals may contribute to the neuropathological processes associated with Cu^{2+} toxicity in Wilson's disease.

Keywords: Cu^{2+}; K^+ channel; Synaptosome; Wilson's disease; Catecholamine release

1. Introduction

Cu^{2+} is an essential trace element involved in many important biological processes. Pathological changes in the levels of this divalent transition metal lead to a variety of clinical pathologies. In particular, mutations in distinct but homologous Cu^{2+} ATPases result in Menkes Syndrome (Cu^{2+} deficiency) and Wilson's disease (Cu^{2+} toxicity) (DiDonato and Sarkar, 1997). In Wilson's disease, accumulation of Cu^{2+} leads to both hepatic pathology and neurological symptoms such as Parkinsonian signs and dystonia. Recently, striatal degeneration as well as damage to the presynaptic terminals of the nigral dopaminergic neurons have been detected in patients with Wilson's disease, which may contribute to their Parkinsonian symptoms (Roh et al., 1994; Jeon et al., 1998). Changes in catecholamine levels in different brain regions have also been reported in Cu^{2+}-deficient (Prohaska and Bailey, 1993; Prohaska and Bailey, 1994) and Cu^{2+}-toxicity animal models (Saito et al., 1996). In vitro, Cu^{2+} causes depolarization of neurons (Dreifuss et al., 1969; Weinreich and Wonderlin, 1987), blocks K^+ and Na^+ channels in axons (Arhem, 1980), inhibits synaptosomal uptake of monoamine transmitters which may secondarily increase transmitter efflux (Komulainen and Tuomisto, 1981; Tuomisto and Komulainen, 1983), and stimulates leutenizing hormone-releasing hormone release from median eminence explants (Barnea and Colombani-Vidal, 1984). During the course of studying the ability of divalent transition metals to modulate the presynaptic NMDA receptors on catecholaminergic nerve terminals (Wang and Thukral, 1996), we observed a strong effect of some of these heavy metals on catecholamine release. In this report, I show that Cu^{2+} causes a massive Ca^{2+}-dependent release of neurotransmitters from isolated CNS dopaminergic and noradrenergic nerve terminals.

2. Materials and methods

2.1. Materials

All drugs and chemicals were obtained from the Sigma (St. Louis, MO, USA). All chemicals were of reagent

* Corresponding author. Tel.: +1-617-636-3643; Fax: +1-617-636-2413; E-mail: jwang@opal.tufts.edu

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00275-7

Table 1
Divalent metal ions induce catecholamine release from [^{3}H]noradrenaline-loaded hippocampal and [^{3}H]dopamine-loaded striatal synaptosomes

Net release	+ Cu^{2+} 100 μM	+ Zn^{2+} 300 μM	+ Ni^{2+} 1 mM	+ 4-Aminopyridine 500 μM
[^{3}H]Noradrenaline	20.1 ± 5.3% (5)	5.7 ± 2.6% (3)	8.0 ± 2.1% (5)	18.5 ± 4.1% (5)
[^{3}H]Dopamine	19.1 ± 3.1% (4)	4.5 ± 1.5% (4)	3.9% (1)	22.6 ± 8.2% (3)

Hippocampal synaptosomes preloaded with [^{3}H]noradrenaline and striatal synaptosomes preloaded with [^{3}H]dopamine were superfused for 5 min with buffer containing the indicated concentrations of divalent metal ions or 4-aminopyridine, and the released transmitter was quantitated as described in Section 2. Results are expressed as percent of total transmitter pool (mean ± S.D. (*n*)). Net release is derived by subtracting basal release in the absence of the metal ions (6.8 ± 1.5% (5) for [^{3}H]noradrenaline; 7.5 ± 1.5% (4) for [^{3}H]dopamine) from the release in their presence.

grade. [^{3}H]noradrenaline and [^{3}H]dopamine were obtained from NEN-Dupont (Boston, MA, USA).

2.2. Preparation of synaptosomes

Synaptosomes were prepared as described in Wang et al. (1992). Briefly, P_2 fractions were prepared from hippocampus and striatum of adult male Sprague–Dawley rats (150–250 g). Synaptosomes were resuspended in incubation buffer containing the following (in mM): NaCl, 140; $MgCl_2$, 1; $CaCl_2$, 1; KCl, 5; $NaHCO_3$, 5; NaH_2PO_4, 1.2; glucose, 10; HEPES, 10 (pH 7.4); ascorbic acid, 0.2; and pargyline, 0.02.

2.3. Transmitter release assay

Hippocampal synaptosomes were loaded with [^{3}H]noradrenaline (0.007 μM, 70.2 Ci/mmol), and striatal synaptosomes were loaded with [^{3}H]dopamine (0.015 μM, 35.1 Ci/mmol), for 10 min at 37°C. The loaded synaptosomes were placed on Whatman GF/B filters (100–150 μg total protein on each filter) in a superfusion chamber, washed for 10 min, and superfused with buffer containing the indicated compounds at a nominal flow rate of 3 ml/min under unit gravity. Cupric chloride (all other divalent heavy metals ions tested were also as chloride salt) was used for all the experiments (preliminary experiments with cupric sulfate gave the same results). Superfusion fractions were collected every min for 5 min or as a single 5 min fraction. Labeled transmitter in the superfusate was quantitated by liquid scintillation spectroscopy. The data are expressed as percent of the total transmitter pool, defined as counts retained on the filter plus counts in the collected superfusate. Net release is derived by subtracting the basal spontaneous release from the drug-induced release, and shown as mean ± S.D. as indicated.

2.4. Statistics

Statistical tests were performed with Student's *t*-test as indicated, and significance is defined as $p < 0.05$.

3. Results

3.1. Certain divalent heavy metal ions induce catecholamine release from brain synaptosomes

Synaptosomes prepared from rat hippocampus or striatum and loaded with [^{3}H]noradrenaline or [^{3}H]dopamine respectively were placed under superfusion at a flow rate of 3 ml/min to assay for transmitter release upon exposure to divalent transition metal cations. Under these conditions, transmitter reuptake into the filter-trapped synaptosomes is not significant, as confirmed by the lack of effect of the noradrenaline reuptake inhibitor desipramine (1 μM) on the basal [^{3}H]noradrenaline release (95% of basal release; $n = 2$). Inclusion of 100 μM Cu^{2+}, 300 μM Zn^{2+}, or 1 mM Ni^{2+} in the superfusion buffer significantly increased transmitter release from both noradrenergic and dopaminergic nerve terminals during 5 min of superfusion (Table 1), while Cd^{2+} (see Table 2) and Co^{2+} (data not shown) had no effect at 1 mM. Cu^{2+} was the most effective among the transition metals tested, the

Table 2
Effects of removal of Ca^{2+} or addition of Cd^{2+} on heavy metal-induced noradrenaline release from hippocampal synaptosomes

Net release		+ Ni^{2+} 1 mM	+ Zn^{2+} 0.3 mM
[^{3}H]Noradrenaline	Ca^{2+} 1 mM	11.7 ± 3.0% (3)	4.8% (2)
	Ca^{2+} 0 mM	0.3 ± 0.2% (3)	0.6% (2)
	Ca^{2+} 1 mM + Cd^{2+} 0.3 mM	2.3% (2)	0.3% (1)

Hippocampal synaptosomes preloaded with [^{3}H]noradrenaline were superfused for 2 min with buffer containing 1 mM Ca^{2+}, 0 mM Ca^{2+}, or 1 mM Ca^{2+} plus 300 μM Cd^{2+}, followed by exposure to the same buffer containing in addition 1 mM Ni^{2+} or 0.3 mM Zn^{2+} for 5 min. Transmitter released during the 5 min period was quantitated as described in Section 2. Results are presented as mean percent of total transmitter pool ± S.D. (*n*). Basal release for the three pretreatment conditions are respectively: 6.6 ± 1.8%, 4.2 ± 1.2%; and 6.2% of the total transmitter pool.

magnitude of its effect on inducing transmitter release from both types of synaptosomes was similar to that of depolarization with 500 μM 4-aminopyridine or 30 mM KCl (Table 1 and Fig. 3) over the 5 min period.

3.2. Cu^{2+}-induced transmitter release is concentration-dependent

The effect of Cu^{2+} was concentration-dependent (Fig. 1), although saturation was not readily achieved even at the highest concentration tested, particularly for the dopaminergic synaptosomes. Indeed, the shape of the concentration–response curves was different for release from the two types of synaptosomes. Thus, Cu^{2+}-induced dopamine release occurred over a much wider concentration range than Cu^{2+}-induced noradrenaline release, with the former detectable at 0.1 μM Cu^{2+} while an equivalent effect for the latter required almost 10 μM Cu^{2+}. Finally, concentration-dependent induction of release by Cu^{2+} and by Ni^{2+} was also observed in olfactory bulb synaptosomes preloaded with [^{3}H]noradrenaline, to a similar extent as with release from hippocampal synaptosomes (data not shown).

3.3. Transmitter release induced by divalent heavy metal ions requires ca^{2+} influx

The ability of Cu^{2+} to induce release from both noradrenergic and dopaminergic synaptosomes required extracellular Ca^{2+}, as the effect was completely blocked by the removal of Ca^{2+} from the superfusion buffer (Fig. 2). A similar requirement for extracellular Ca^{2+} was observed for noradrenaline release from hippocampal synaptosomes induced by 1 mM Ni^{2+} or 300 μM Zn^{2+} (Table 2). Furthermore, 300 μM Cd^{2+}, which had no effect by itself on noradrenaline release, blocked the effects of Zn^{2+} and Ni^{2+} (Table 2) and of Cu^{2+} (net release induced by Cu^{2+} was 7.6% of total in the absence of Cd^{2+} and 0.3% of total in the presence of Cd^{2+}; $n = 2$). Thus, the effect of Cu^{2+} on transmitter release requires Ca^{2+} influx into the catecholaminergic nerve terminals.

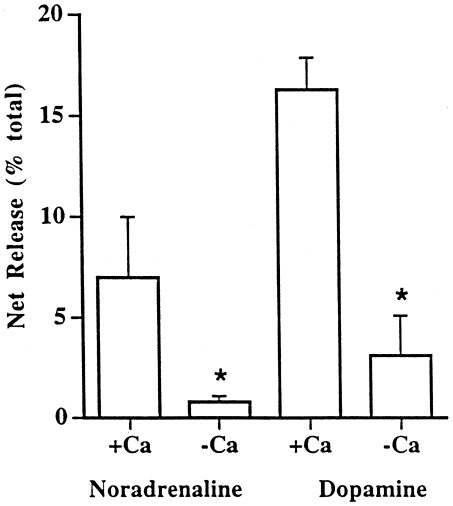

Fig. 2. Ca^{2+}-dependency of the Cu^{2+}-induced transmitter release. [^{3}H]noradrenaline-loaded hippocampal and [^{3}H]dopamine-loaded striatal synaptosomes were superfused for 2 min with buffer containing 1 mM Ca^{2+} (+Ca) or with buffer with no added Ca^{2+} (−Ca), then exposed to 30 μM Cu^{2+} in the respective buffers for 5 min. Transmitter released during the 5 min period was quantitated as described in Section 2. Results are presented as mean ± S.D. ($n = 4$ for [^{3}H]noradrenaline; $n = 3$ for [^{3}H]dopamine). The basal release was, in the presence or absence of Ca^{2+}, respectively: 5.7 ± 1.6% and 3.7 ± 0.5% for [^{3}H]noradrenaline; and 7.9 ± 2.9% and 4.0 ± 0.6% for [^{3}H]dopamine. *Significantly different from the +Ca condition, $p < 0.05$, Student's *t*-test.

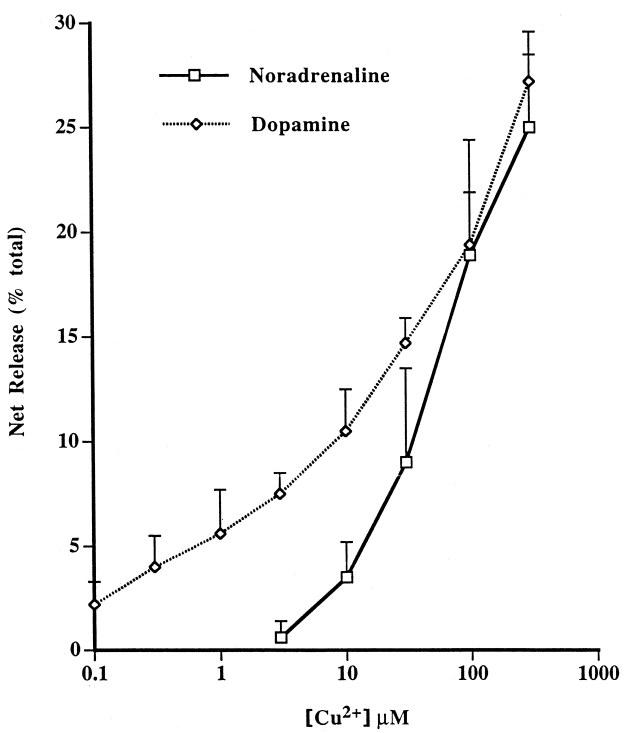

Fig. 1. Concentration–response curves for Cu^{2+}-induced transmitter release from superfused [^{3}H]noradrenaline-loaded hippocampal and [^{3}H]dopamine-loaded striatal synaptosomes. Synaptosomes were exposed to the indicated concentrations of Cu^{2+} for 5 min, and the released transmitter was quantitated as described in Section 2. Results are presented as mean ± S.D. ($n = 3$–5).

3.4. Cu^{2+}-induced transmitter release is slower than that induced by K^+ or 4-aminopyridine

The time course of the Cu^{2+}-induced transmitter release was compared with that induced by 4-aminopyridine and high K^+ (Fig. 3). As expected, direct depolarization with high K^+ resulted in the most rapid transmitter release, with a peak at substantially less than 1 min (the gravity-based superfusion system used here shows an apparent peak at 30 s (not shown), but a fast superfusion system reveals a sub-second time course (Turner et al. (1992)). 4-Aminopyridine, by contrast, showed a slower time course, peaking at 1–2 min and then declining. The time

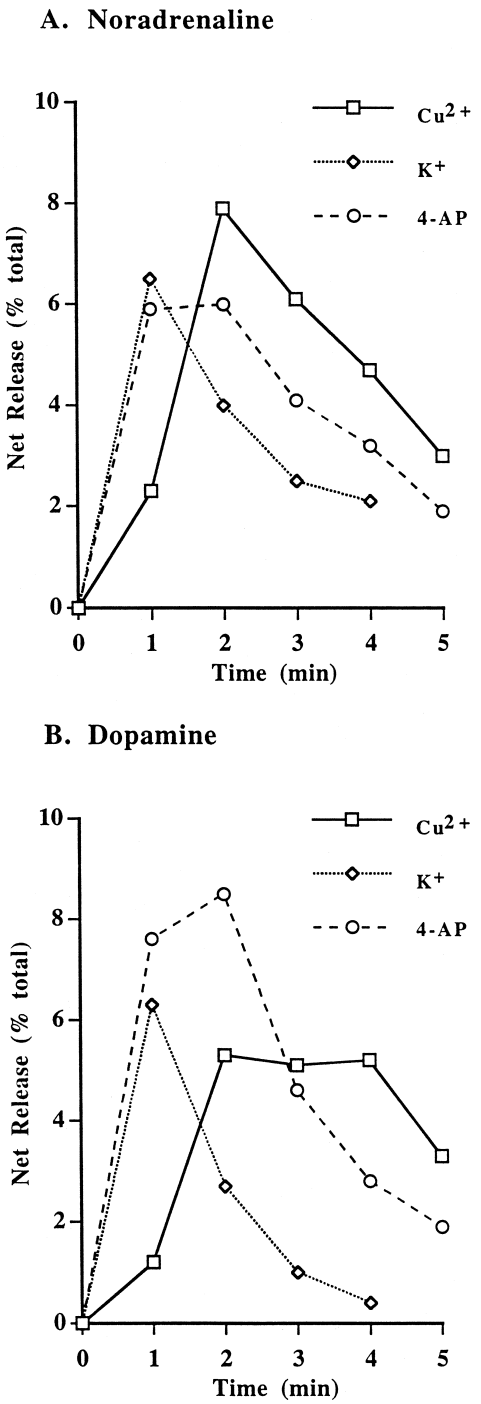

Fig. 3. Time course of transmitter release evoked by Cu^{2+}, 4-aminopyridine (**4-AP**) and high K^+. (A) [^{3}H]noradrenaline-loaded hippocampal or (B) [^{3}H]dopamine-loaded striatal synaptosomes were superfused with 100 μM Cu^{2+}, 500 μM 4-aminopyridine, or 30 mM K^+. Fractions were collected every min, and the released transmitter was quantitated as described in Section 2. Results are presented as means of 2 (K^+) or 3 (Cu^{2+} and 4-aminopyridine) independent experiments. S.D. values were all within 20% of the means and are not shown for the sake of clarity.

course of Cu^{2+}-induced noradrenaline release (averaged from three independent experiments) was even slower in onset than that of 4-aminopyridine, with a peak response at 2 min of superfusion (Fig. 3A). After the peak, however, the decline in the time course appears similar that of 4-aminopyridine. A similar time course with a delayed

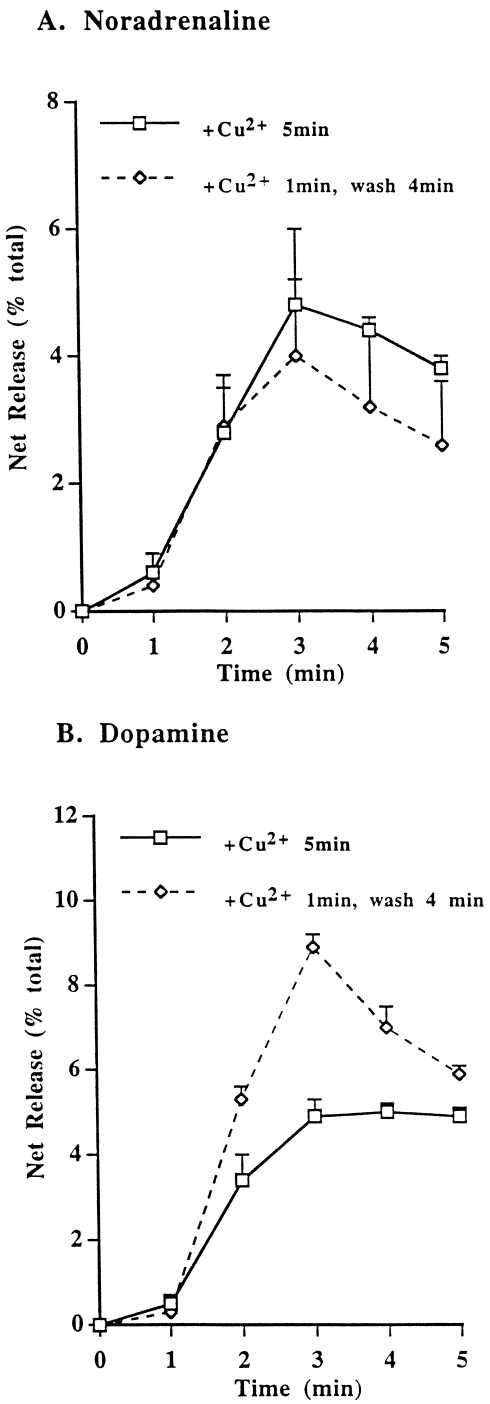

Fig. 4. Reversibility of the effect of Cu^{2+} on transmitter release. (A) [^{3}H]noradrenaline-loaded hippocampal and (B) [^{3}H]dopamine-loaded striatal synaptosomes were superfused with 100 μM Cu^{2+} either continuously for 5 min (+Cu^{2+} 5 min), or for only 1 min followed by 4 min of Cu^{2+}-free buffer (+Cu^{2+} 1 min, wash 4 min). Fractions were collected every min, and the released transmitter was quantitated as described in Section 2. Results are presented as mean ± S.D. of triplicate samples in a representative experiment. Similar results were obtained in two additional independent experiments (not shown).

Table 3
Effects of tetrodotoxin on Cu^{2+}- and 4-aminopyridine-induced transmitter release from [^{3}H]noradrenaline-loaded hippocampal and [^{3}H]dopamine-loaded striatal synaptosomes

Net release	+ Cu^{2+}	+ Cu^{2+} + tetrodotoxin	+ 4-Aminopyridine	+ 4-Aminopyridine + tetrodotoxin
[^{3}H]Noradrenaline ($n = 5$)	5.0 ± 1.8%	2.3 ± 0.9%*	16.6 ± 0.6%	5.5 ± 1.5%*
[^{3}H]Dopamine ($n = 3$)	13.9 ± 1.8%	9.7 ± 1.6%	15.9 ± 2.9%	12.2 ± 1.6%

Hippocampal synaptosomes preloaded with [^{3}H]noradrenaline and striatal synaptosomes preloaded with [^{3}H]dopamine were superfused for 2 min with buffer containing 3 μM tetrodotoxin, followed by exposure to the same buffer containing in addition 30 μM Cu^{2+} or 200 μM 4-aminopyridine for 5 min. Transmitter released during the 5 min period was quantitated as described in Section 2. Results are presented as mean percent of total transmitter pool ± S.D. (basal release for [^{3}H]noradrenaline: 7.9 ± 2.0%, + tetrodotoxin: 6.4 ± 1.7%; basal release for [^{3}H]dopamine: 7.7 ± 1.6%, + tetrodotoxin: 7.8 ± 2.6%).
* Significantly different from condition without tetrodotoxin, $p < 0.01$, paired Student's *t*-test.

onset followed by a decline was observed for noradrenaline release induced by Zn^{2+} and Ni^{2+} (data not shown). Thus, the time course of Cu^{2+}-induced noradrenaline release appears somewhat similar to that of 4-aminopyridine, with the major difference being a slower onset in the former. Cu^{2+}-induced dopamine release also lagged behind that of 4-aminopyridine and high K^+, but was then maintained at the peak for at least 3 min (Fig. 3B). Thus, Cu^{2+}-induced dopamine release is not only slow in onset but also more sustained.

3.5. Transient exposure to Cu^{2+} causes sustained transmitter release

I also examined the effect of a transient exposure to Cu^{2+} on transmitter release from the two types of superfused synaptosomes. Preloaded synaptosomes were exposed to Cu^{2+} for 1 min under superfusion, followed by 4 min of superfusion with Cu^{2+}-free buffer. Interestingly, the increase in noradrenaline release was similar whether Cu^{2+} was present only during the first min or throughout the 5 min of superfusion (Fig. 4A shows a representative experiment, the same result was obtained in two additional independent experiments). Surprisingly, 1 min of exposure to Cu^{2+} caused even more dopamine release from striatal synaptosomes during the subsequent 4 min than that caused by continued exposure to Cu^{2+} (Fig. 4B shows a representative experiment, the same result was obtained in two additional independent experiments). Thus, the effect of Cu^{2+} on transmitter release is not reversible by washout, and in the case of dopamine release removal of Cu^{2+} from the superfusion buffer after an initial exposure actually enhanced the subsequent effect.

3.6. Sensitivity of Cu^{2+}-induced transmitter release to tetrodotoxin

Finally, I examined whether Cu^{2+}-induced transmitter release involved the voltage-gated Na^+ channels by determining the effects of the specific channel blocker tetrodotoxin. 4-aminopyridine-induced noradrenaline release was as expected sensitive to tetrodotoxin (Table 3), as was Cu^{2+}-induced noradrenaline release (Table 3). However, both the 4-aminopyridine- and the Cu^{2+}-induced dopamine release were not significantly affected by tetrodotoxin treatment (Table 3). Thus, the effect of Cu^{2+} on transmitter release, certainly in the case of noradrenaline, requires the activation of tetrodotoxin-sensitive voltage-gated Na^+ channels.

4. Discussion

In a superfusion system containing intact nerve terminals isolated from their neuronal cell bodies, Cu^{2+} at μM concentrations induce transmitter release from hippocampal and olfactory bulb synaptosomes preloaded with [^{3}H]noradrenaline, and from striatal synaptosomes preloaded with [^{3}H]dopamine. Under these conditions, the uptake of [^{3}H]noradrenaline by the hippocampal and olfactory synaptosomes is completely inhibited by the selective noradrenergic uptake inhibitor desipramine (Wang et al., 1992), while the uptake of [^{3}H]dopamine by the striatal synaptosomes is inhibited by a selective dopaminergic uptake inhibitor 1-[2-(bis[4-fluorophenyl]-methoxy)ethyl]-4-[3-phenylpropyl]-piperazine) (GBR12909) (unpublished data). Thus, the effect of Cu^{2+} is likely to be exerted locally and directly on nerve terminals of the locus ceruleus noradrenergic neurons, which innervate the hippocampus and the olfactory bulb among other brain regions, and on nerve terminals of the nigral dopaminergic neurons, which innervate mainly the striatum. The effect of Cu^{2+} is similar in magnitude to that of depolarization with high K^+ or 4-aminopyridine, suggesting a direct action on the majority (rather than a subpopulation) of the catecholaminergic nerve terminals in these brain regions. Zn^{2+} and Ni^{2+}, but not Cd^{2+} or Co^{2+}, also induced transmitter release but are much less effective than Cu^{2+}, suggesting that there is some selectivity in the mechanism of action of these transition heavy metals. Importantly, transmitter release induced by Cu^{2+} and the other effective transition metals is dependent on extracellular Ca^{2+}, indicating a requirement for Ca^{2+} influx and the origin of the released transmitters from the synaptic vesicular pool. Moreover, Cd^{2+}, which by itself has no effect on transmitter release but is known to block voltage-gated Ca^{2+} channels, was

able to prevent the noradrenaline release induced by the transition metals, indicating an activation of the voltage-gated Ca^{2+} channels. This requirement for Ca^{2+} influx also rules out the involvement of the catecholamine transporters, which have been previously shown to be inhibited by Cu^{2+} and may therefore contribute to an apparent increase in transmitter release under static incubation conditions (Komulainen and Tuomisto, 1981; Tuomisto and Komulainen, 1983). Further support that this mechanism did not play a role in the results reported here is the lack of effect of the uptake blocker desipramine on the basal [^{3}H]noradrenaline release, as these synaptosomes were placed under superfusion condition that effectively eliminated contribution from the reuptake process. Thus, the effect of Cu^{2+} on catecholamine release is ultimately dependent on an opening of the voltage-gated Ca^{2+} channels, influx of Ca^{2+}, and activation of the Ca^{2+}-dependent synaptic vesicular secretory machinery.

The mechanism(s) by which Cu^{2+} activates the voltage-gated Ca^{2+} channels to induce catecholamine release from nerve terminals is unknown. Cu^{2+}-induced noradrenaline release is as robust as that induced by 4-aminopyridine and high K^+, is dependent on Ca^{2+} influx through voltage-gated Ca^{2+} channels, and involves activation of voltage-gated Na^+ channels (as revealed by its the sensitivity to tetrodotoxin). The tetrodotoxin sensitivity makes it unlikely that Cu^{2+} is directly acting on the Ca^{2+} channels. Cu^{2+} has been reported to depolarise neuronal soma (Dreifuss et al., 1969; Weinreich and Wonderlin, 1987), and to blocks K^+ channels in axons (Arhem, 1980). These results suggest that one possible mechanism by which Cu^{2+} can induce transmitter release may be modulation of the nerve terminal membrane potential. Indeed, the ability of 4-aminopyridine to induce transmitter release is due to its inhibition of nerve terminal K^+ channels that maintain the resting membrane potential, thereby activating Na^+ channels and depolarizing the nerve terminal membrane sufficiently to open Ca^{2+} channels (Tibbs et al., 1996). It is also known that Zn^{2+} and Cu^{2+} can block K^+ channels such as the shaker type channels (Q. Lu and C. Miller, Brandeis University; unpublished data and personal communication). The overall similarity of the time course of the Cu^{2+}-induced noradrenaline release to that of 4-aminopyridine, a well-known K^+ channel blocker, suggests that such a mechanism may be at work in the catecholaminergic nerve terminals. However, Cu^{2+}-induced noradrenaline release is slower in onset and is not readily reversible compared to 4-aminopyridine, suggesting that if Cu^{2+} blocks nerve terminal K^+ channels the mechanism of channel blockade must be distinct from that of 4-aminopyridine. Perhaps in common with other divalent cations such as Ba^{2+} (Sihra et al., 1993), Cu^{2+} is also capable of permeating nerve terminal membrane through divalent cation selective channels. Its presumed inhibition of K^+ channels, therefore, may occur intracellularly, thus accounting for the slower time course and the lack of reversibility. Indeed, it appears that Cu^{2+} is more effective as a blocker of the shaker type K^+ channels on the intracellular side relative to the extracellular side (Q. Lu and C. Miller, Brandeis University; unpublished data and personal communication). However, there is as yet no evidence that Cu^{2+} and other divalent metal ions can inhibit K^+ channels from the intracellular side in catecholaminergic nerve terminals. Evidence to support this purely speculative mechanism of action will be difficult to obtain in a heterogeneous system such as the synaptosomal preparation, in which only a fraction of the total population is constituted by catecholaminergic nerve terminals. Indeed, it appears that Ni^{2+}, which can induce catecholamine release, does not affect glutamate release from cerebral cortical synaptosomes (T. Turner, Tufts University; unpublished data and personal communication), suggesting that the robust effect of the transition metals on transmitter release may be limited to the catecholamines. Moreover, Cu^{2+} does not significantly alter the average membrane potential of rat forebrain synaptosomes (Nachshen, 1984), suggesting that the majority of nerve terminals in this preparation (mostly releasing glutamate or GABA) is not sensitive to Cu^{2+} in the manner proposed in this report. Thus, it is possible that Cu^{2+} affects only catecholaminergic nerve terminals (which constitute only a fraction of the isolated nerve terminals), perhaps due to expression of specific types of Cu^{2+}-sensitive K^+ channel in these terminals.

The mechanism underlying Cu^{2+}-induced dopamine release appears more complex than for noradrenaline release, as suggested by differences in their concentration–response curves, time courses, reversibility, and tetrodotoxin sensitivity. The concentration–response curve of Cu^{2+} for dopamine release spans more than three orders of magnitude, yet with no apparent sign of saturation. This is consistent with a multi-phasic response. The time course of the Cu^{2+}-induced dopamine release shows a lag phase similar to that of noradrenaline release, but the peak response appears more sustained. Indeed, 1 min of exposure to Cu^{2+} resulted in higher amounts of dopamine release over the subsequent 4 min than with continuous exposure. Perhaps Cu^{2+} also partially inhibit dopamine release by a different and slower mechanism, an effect that is enhanced only upon prolonged exposure to the heavy metal ion. Interestingly, tetrodotoxin did not significantly suppress either the 4-aminopyridine- or the Cu^{2+}-induced dopamine release (it inhibited 23% of the former and 30% of the latter, but the differences did not reach statistical significance). A previous report showed that tetrodotoxin inhibited only a fraction of the initial 4 min of dopamine release (inhibition of 25%, though it was statistically significant), and not at all the subsequent release, induced by K^+ channel blockade (see Fig. 5 and text in Bowyer and Weiner, 1989). Taken together, these data suggest that the Na^+ channels present in the striatal nerve terminals of the nigral dopaminergic neurons are relatively tetrodotoxin-in-

sensitive. Alternatively, the ability of K^+ channel blockers to induce dopamine release is largely independent of Na^+ channels. Nevertheless, the effect of Cu^{2+} on dopamine release is entirely dependent on extracellular Ca^{2+}, consistent with an action that must ultimately activate the Ca^{2+}-dependent vesicular secretory machinery, as is the case with noradrenaline release. Taken together, data reported here suggest that the final result of exposure to Cu^{2+} is likely nerve terminal membrane depolarization and Ca^{2+}-dependent transmitter release from both types of catecholaminergic nerve terminals. Indeed, the primary effect of Cu^{2+} on the catecholaminergic terminals may be membrane depolarization, which in addition to causing transmitter release can also secondarily inhibit transmitter reuptake. In vivo, therefore, increase in the levels of Cu^{2+} is likely to cause a net increase in transmitter release from the catecholaminergic nerve terminals. Dopamine at higher concentrations is toxic to striatal neurons, an effect that is potentiated by mitochondrial compromise (McLaughlin et al., 1998). Presumably, chronic Cu^{2+} toxicity in Wilson's disease can impair catecholaminergic transmission due to sustained catecholamine transmitter release uncoupled from synaptic activity. Over time, a sustained increase in striatal dopamine levels may lead to neuronal damage and death, thus contributing to the neuropathological manifestations of the disease.

Acknowledgements

the expert technical assistance of J. Lin and C. Canizio is gratefully acknowledged. Supported by grant R01NS30590 from the National Institutes of Health.

References

Arhem, P., 1980. Effects of some heavy metal ions on the ionic currents of myelinated fibres from *Xenopus laevis*. J. Physiol. (London) 306, 219–231.

Barnea, A., Colombani-Vidal, M., 1984. A ligand-specific action of chelated copper on hypothalamic neurons: stimulation of the release of luteinizing hormone-releasing hormone from median eminence explants. Proc. Natl. Acad. Sci. U.S.A. 81, 7656–7660.

Bowyer, J.F., Weiner, N., 1989. K^+ channel and adenylate cyclase involvement in regulation of Ca^{2+}-evoked release of [^{3}H]dopamine from synaptosomes. J. Pharmacol. Exp. Ther. 248, 514–520.

DiDonato, M., Sarkar, B., 1997. Copper transport and its alterations in Menkes and Wilson diseases. Biochim. Biophys. Acta 1360, 3–16.

Dreifuss, J.J., Kelly, J.S., Krnjevic, K., 1969. Effects of copper on cortical neurones. Brain Res. 13, 607–611.

Jeon, B., Kim, J.M., Jeong, J.M., Kim, K.M., Chang, Y.S., Lee, D.S., Lee, M.C., 1998. Dopamine transporter imaging with [^{123}I]-beta-CIT demonstrates presynaptic nigrostriatal dopaminergic damage in Wilson's disease. J. Neurol., Neurosurg. Psychiatry 65, 60–64.

Komulainen, H., Tuomisto, J., 1981. Effect of copper on the uptake and release of monoamines in rat brain synaptosomes. Acta Pharmacol. Toxicol. (Copenh) 48, 205–213.

McLaughlin, B.A., Nelson, D., Erecinska, M., Chesselet, M.F., 1998. Toxicity of dopamine to striatal neurons in vitro and potentiation of cell death by a mitochondrial inhibitor. J. Neurochem. 70, 2406–2415.

Nachshen, D.A., 1984. Selectivity of the Ca binding site in synaptosome Ca channels. J. Gen. Physiol. 83, 941–967.

Prohaska, J.R., Bailey, W.R., 1993. Persistent regional changes in brain copper, cuproenzymes and catecholamines following perinatal copper deficiency in mice. J. Nutr. 123, 1226–1234.

Prohaska, J.R., Bailey, W.R., 1994. Regional specificity in alterations of rat brain copper and catecholamines following perinatal copper deficiency. J. Neurochem. 63, 1551–1557.

Roh, J.K., Lee, T.G., Wie, B.A., Lee, S.B., Park, S.H., Chang, K.H., 1994. Initial and follow-up brain MRI findings and correlation with the clinical course in Wilson's disease. Neurology 44, 1064–1068.

Saito, T., Nagao, T., Okabe, M., Saito, K., 1996. Neurochemical and histochemical evidence for an abnormal catecholamine metabolism in the cerebral cortex of the Long–Evans Cinnamon rat before excessive copper accumulation in the brain. Neurosci. Lett. 216, 195–198.

Sihra, T.S., Piomelli, D., Nichols, R.A., 1993. Barium evokes glutamate release from rat brain synaptosomes by membrane depolarization: involvement of K^+, Na^+, and Ca^{2+} channels. J. Neurochem. 61, 1220–1230.

Tibbs, G.R., Dolly, J.O., Nicholls, D.G., 1996. Evidence for the induction of repetitive action potentials in synaptosomes by K^+-channel inhibitors: an analysis of plasma membrane ion fluxes. J. Neurochem. 67, 389–397.

Tuomisto, J., Komulainen, H., 1983. Ca-dependence of accumulation of monoamines into synaptosomes and its inhibition by copper. Acta Pharmacol. Toxicol. (Copenh) 53, 193–199.

Turner, T.J., Adams, M.E., Dunlap, K., 1992. Calcium channels coupled to glutamate release identified by omega-Aga-IVA. Science 258, 310–313.

Wang, J.K.T., Andrews, H., Thukral, V., 1992. Presynaptic glutamate receptors regulate noradrenaline release from isolated nerve terminals. J. Neurochem. 58, 204–211.

Wang, J.K.T., Thukral, V., 1996. Presynaptic NMDA receptors display physiological characteristics of homomeric complexes of NR1 subunits that contain the exon 5 insert in the N-terminal domain. J. Neurochem. 66, 865–868.

Weinreich, D., Wonderlin, W.F., 1987. Copper activates a unique inward current in molluscan neurones. J. Physiol. (London) 394, 429–443.